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OF OTOLOGY, RHINOLOGY & LARYNGOLOGY

ANNALS OF HEAD & NECK MEDICINE & SURGERY

MICROBIOLOGY OF WOUND INFECTION AFTER HEAD AND NECK CANCER SURGERY

CLINICAL APPLICATIONS OF EVOKED ACOUSTIC EMISSIONS

RECURRENT TONSILLITIS

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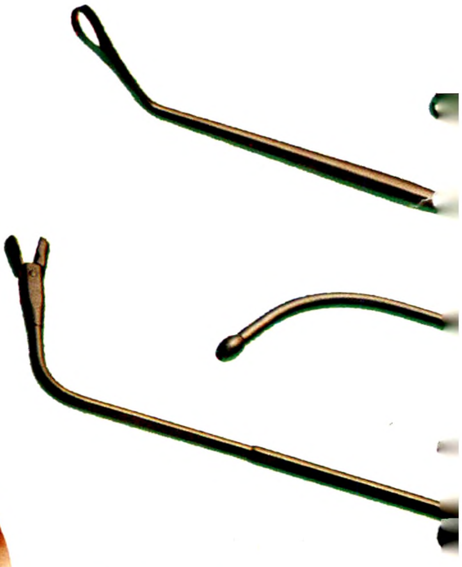

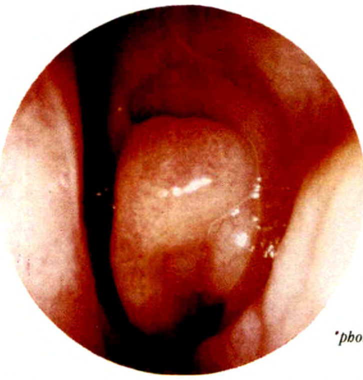

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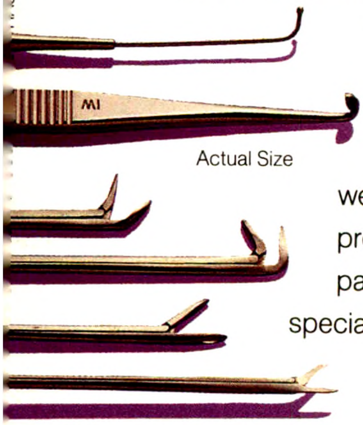

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MICROBIOLOGY OF WOUND INFECTION AFTER HEAD AND NECK CANCER SURGERY

ITZHAK BROOK, MD, MSc RONALD HIROKAWA, MD

BETHESDA, MARYLAND

Specimens of pus were obtained from 24 patients who developed postoperative wound infection after head and neck cancer surgery. Aerobic bacteria only were isolated in two instances (8%), anaerobic bacteria only in one (4%), and mixed aerobic and anaerobic bacteria in 21 (88%). A total of 146 isolates were recovered (66 aerobic and 80 anaerobic), an average of six isolates per specimen (2.7 aerobic and 3.3 anaerobic). The most frequently recovered isolates were *Peptostreptococcus* sp, *Staphylococcus aureus*, *Bacteroides* sp, *Fusobacterium*, and enteric gram-negative rods. Twenty-two isolates recovered from 17 wounds (71%) produced β-lactamase. These included all five isolates of *S aureus* and nine of 17 (53%) of the *Bacteroides melaninogenicus* group. The polymicrobial aerobic/anaerobic nature of postoperative wound infections after head and neck cancer surgery and the presence of β-lactamase–producing bacteria may have important implications for the management of these infections.

KEY WORDS — *Bacteroides* species, β-lactamase–producing bacteria, cancer, microbiology, *Staphylococcus aureus*, wounds.

INTRODUCTION

Wound infection frequently occurs after head and neck surgery for malignancy. These infections have been shown to be due to mixed aerobic and anaerobic bacteria.[1-3] The recovery of such flora is not surprising, since the oral cavity is colonized by mixed aerobic and anaerobic bacteria that reach the numbers of 10^7 to 10^8 aerobes and 10^8 to 10^9 anaerobes per milliliter of saliva.[4] Until recently, most of these organisms were susceptible to penicillins and first generation cephalosporins. However, within the last decade, many of these bacteria have shown increased resistance to these drugs because of their ability to produce the enzyme β-lactamase.[5] Furthermore, the presence of β-lactamase–producing bacteria (BLPB) has been associated with the failure of penicillin to eradicate oral infection.[6,7] This study reports our experience in analyzing the aerobic and anaerobic flora and the presence of BLPB in postoperative wound infection after head and neck surgery.

PATIENTS AND METHODS

Patients. Specimens of pus were obtained from 24 patients (15 men and nine women) who had undergone major head and neck cancer surgery in the Naval Medical Center (Bethesda, Md) and developed wound infections between June 1978 and June 1984. During that period about 400 patients at the same hospital had major head and neck cancer surgery. The 24 patients' ages ranged between 27 and 68 years (median age, 49 years). Antimicrobial prophylaxis of one intramuscular injection of a first generation cephalosporin (cefazolin sodium) prior to surgery was given to 13 of these patients. None of the other 11 patients received any antimicrobial prophylaxis.

Culture Methods. Samples for culture were obtained from the infected area when feasible by the aspiration of pus (after cleansing of the area with povidone-iodine) or by swabbing of the wound. Aspirated specimens were transported by syringe and needle capped with a rubber stopper. Swabs were placed in an anaerobic transport medium (Port-A-Cul, BBL Microbiological Systems, Becton Dickinson, Cockeysville, Md). The specimens were transported to the microbiology laboratory, where they were inoculated into media supporting aerobic and anaerobic organisms. The time between specimen collection and inoculation never exceeded 30 minutes. Gram stain preparations were made of all specimens.

Sheep blood, chocolate, and MacConkey's agar plates were inoculated for aerobes. The plates were incubated at 37°C aerobically (MacConkey's) or under 5% carbon dioxide and were examined at 24 and 48 hours. For anaerobes, the material was plated on the bench onto prereduced vitamin K1–enriched brucella blood agar, an anaerobic blood agar plate containing kanamycin and vancomycin, an anaerobic blood plate containing phenylethyl alcohol, and an enriched thioglycolate broth (containing hemin, sodium bicarbonate, and vitamin K1).[8] The plates were incubated in GasPak jars (BBL Microbiological Systems) and examined at 48 and 96 hours. The thioglycolate broth was incubated for 14 days. Anaerobes were identified by techniques described previously.[9] Aerobic bacteria were identified by conventional methods.[10] β-Lactamase activity was determined on all isolates by

---

From the Departments of Pediatrics (Brook) and Surgery (Brook, Hirokawa), Uniformed Services University of the Health Sciences, Bethesda, Maryland. The opinions and assertions contained herein are the private ones of the writers and are not to be construed as official or reflecting the views of the Department of Defense, the Navy Department, or the naval service at large.

REPRINTS — Itzhak Brook, MD, MSc, Armed Forces Radiobiology Research Institute, Bethesda, MD 20814-5145.

AEROBIC, FACULTATIVE ANAEROBIC, AND ANAEROBIC BACTERIA ISOLATED FROM 24 PATIENTS WITH INFECTED POSTOPERATIVE NECK WOUNDS

Aerobic and facultative anaerobic isolates	
Staphylococcus aureus	5 (5)
Staphylococcus epidermidis	2 (1)
Streptococci	
α-Hemolytic	13
Group A β-hemolytic	3
Non–group A β-hemolytic	6
γ-Hemolytic	4
Enterococci	2
Microaerophilic	4
Corynebacterium sp	2
Branhamella catarrhalis	3 (1)
Eikenella corrodens	3
Klebsiella pneumoniae	4 (2)
Pseudomonas aeruginosa	1 (1)
Serratia marcescens	2
Enterobacter aerogenes	4
Proteus mirabilis	3
Hemophilus influenzae	1
Hemophilus parainfluenzae	2
Candida sp	2
Total number aerobes	66 (10)
Anaerobic isolates	
Peptostreptococcus sp	22
Veillonella sp	6
Bifidobacterium sp	2
Lactobacillus sp	1
Propionibacterium acnes	4
Eubacterium sp	2
Fusobacterium sp	2
Fusobacterium nucleatum	7
Fusobacterium necrophorum	3
Bacteroides sp	3
Bacteroides melaninogenicus	11 (5)
Bacteroides asaccharolyticus	2 (2)
Bacteroides intermedius	4 (2)
Bacteroides oralis	3 (2)
Bacteroides disiens	2
Bacteroides thetaiotamicron	1 (1)
Bacteroides oris-buccae	4
Bacteroides uniformis	1
Total number anaerobes	80 (12)

Numbers in parentheses indicate numbers of isolates that produced β-lactamase.

the methodology of chromogenic cephalosporin analog 83/312.[11]

RESULTS

Bacteria were recovered in all 24 specimens. Polymicrobial flora were found in all instances in which the number of isolates varied from two to eight. Aerobic bacteria only were isolated in two instances (8%), anaerobic bacteria only in one (4%), and mixed aerobic and anaerobic bacteria in 21 (88%). No differences were noted in the recovery of organisms and the susceptibility of the organisms between the group of patients who received cefazolin sodium prophylaxis and those who did not.

A total of 146 isolates (six per specimen) were recovered (see Table): 66 aerobes (2.7 per specimen) and 80 anaerobes (3.3 per specimen). The most frequent aerobic isolates, in descending order of frequency, were α-hemolytic streptococci, non–group A β-hemolytic streptococci, and *Staphylococcus aureus*. The most common anaerobic isolates were *Peptostreptococcus* sp, *Bacteroides* sp, and *Fusobacterium* sp.

β-Lactamase activity was detected in 22 isolates recovered from 17 wounds (71%) (see Table). These included all isolates of *S aureus* and nine of 17 isolates (53%) of the *Bacteroides melaninogenicus* group (*B melaninogenicus*, *Bacteroides asaccharolyticus*, and *Bacteroides intermedius*).

DISCUSSION

The results of this study confirm previous data documenting the polymicrobial aerobic/anaerobic flora of postoperative wound infections after head and neck cancer surgery. A synergistic relationship has been shown to exist between these organisms,[12,13] which may make them more virulent and more difficult to eradicate.

The presence of BLPB in over two thirds of our patients may affect the choice of antimicrobial agents for prophylaxis and therapy to prevent and cure these infections. β-Lactamase–producing bacteria may shield other organisms that are susceptible to penicillin from the activity of that drug.[14] The ability of BLPB to protect penicillin-sensitive microorganisms has been demonstrated in vitro[15,16] and in vivo.[17] Several studies[3,18] have illustrated the superiority of prophylactic antimicrobial agents that are effective against aerobic and anaerobic BLPB over agents that are ineffective against these organisms. A recent study[18] demonstrated the superiority of the combination of clindamycin phosphate and gentamicin sulfate over cefazolin sodium in preventing postoperative infection after head and neck surgery (7% versus 33% infection rate). Cefoxitin sodium, which is effective against β-lactamase–producing *Bacteroides* sp, *S aureus*, and many gram-negative enteric bacteria, also was found effective in prevention of these infections.[19] The rate of infection in nontreated patients was 80%, compared to 15% in those receiving cefoxitin.

β-Lactamase–producing bacteria can emerge rapidly in patients who receive penicillin therapy.[20] Furthermore, such organisms are prevalent in the hospital environment. The presence of polymicrobial flora that includes BLPB in postoperative wounds after head and neck cancer surgery warrants the use of antimicrobial agents that are effective against these organisms. These include 1) single therapy with cefoxitin, 2) a combination of clindamycin phosphate, metronidazole hydrochloride, or cefoxitin sodium plus an aminoglycoside, or 3) ticarcillin disodium plus clavulanic acid.

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THE INTERNATIONAL SYMPOSIUM ON CLINICAL AND BASIC OTOLOGY

The International Symposium on Clinical and Basic Otology will be held June 4-6, 1990, in Beijing, People's Republic of China. The abstract deadline is December 31, 1989. For further information, contact the Beijing Institute of Otorhinolaryngology, Chan Liu, MD, Professor and Director, 17 Hougou Lane, Beijing 1000005, People's Republic of China.

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CLINICAL APPLICATIONS OF EVOKED ACOUSTIC EMISSIONS: RESULTS IN NORMALLY HEARING AND HEARING-IMPAIRED SUBJECTS

PIERRE BONFILS, MD, PHD   ALAIN UZIEL, MD, PHD

MONTPELLIER, FRANCE

Click-evoked otoacoustic emissions were recorded in a group of normally hearing subjects (n = 52 ears) and a group of patients demonstrating several common types of sensorineural hearing loss (n = 85 ears) in order to study the clinical applicability of acoustic emissions. In the normally hearing population, all ears demonstrated acoustic emissions that could be elicited at intensities below the normal subjective threshold for the sequence of clicks (mean, −5.2 dB hearing level [HL]). Sixty-seven percent of the normal ears showed long (>20 ms) click-evoked emissions, and spectral analysis showed a broadband spectral component with several narrowband frequency peaks. In patients with sensorineural hearing loss, the incidence of acoustic emissions decreased and the detection threshold increased linearly with increasing threshold. No emissions were elicited from ears exhibiting subjective click thresholds >35 dB HL. Hearing-impaired ears showed a significantly higher proportion of short duration emissions and a significant reduction in the mean number of narrowband frequency peaks in comparison to the controls. Therefore, acoustic emissions can be used as a reliable technique for objective study of normal micromechanical activity within the cochlea and for detection of subtle changes in cochlear disease. However, they have not been demonstrated to be useful in differentiating cochlear disease.

KEY WORDS — cochlea, evoked acoustic emissions, hearing loss.

INTRODUCTION

The existence of acoustic emissions from within the human cochlea was predicted many years ago.[1] Kemp[2] was the first to provide evidence that acoustic emissions can be detected in the human ear canal after stimulation with clicks and short duration tone bursts. Using an acoustic probe consisting of a miniature microphone and a sound source sealed in the external ear canal, he recorded an acoustic response beginning 5 ms following the stimulus onset and lasting for more than 10 ms. His findings since have been confirmed by other investigators in humans,[3-6] laboratory primates,[7] and small mammals.[8-10] Further experiments indicated that these evoked acoustic emissions (EAEs) were cochlear in origin and could be interpreted as a release of acoustic energy into the ear canal from the cochlea transmitted through the ossicular chain and the eardrum.[11]

Evoked acoustic emissions proved to be extremely interesting from a theoretical point of view and have been used extensively to elucidate some of the basic problems in cochlear physiology, leading to the concept of "active processes"[12] within the cochlea. Moreover, this technique also has held some promise as an objective and noninvasive audiologic procedure.[2,13-18] The aim of the present study was to determine the clinical applicability of EAEs as objective indicators of cochlear disease. The basic parameters of EAEs in terms of duration, detection threshold, and frequency content were analyzed in a population of 52 ears from normally hearing subjects and a population of 85 ears with various types and degrees of sensorineural hearing loss.

MATERIALS AND METHODS

Acoustic Probe. Evoked acoustic emissions recordings were obtained with a small acoustic probe similar to that previously described by Johnsen and Elberling.[13] This probe incorporated a small microphone (Knowles BT 1751) and a miniature earspeaker (Knowles BK 2615). Both components of the probe were embedded in a Teflon block that was enclosed in a short plastic speculum forming a conical tip at the microphone input end. In order to avoid excessive acoustic coupling between the microphone and the earspeaker, the earspeaker was connected to the tip of the probe by a polyvinyl tube 1 mm in diameter. The overall length of the probe was 3.5 cm and the weight of the probe was approximately 20 g. The probe was sealed into the ear canal by an impedance probe protector and needed no external support. Acoustic calibration of the probe was carried out in a standard 2-cm^3 coupler (Bruel & Kjaer DB 0138) connected to a 2.5-cm (1 in) condenser microphone (Bruel & Kjaer 4131) using a Bruel & Kjaer 2606 measuring amplifier.

Recording of Evoked Acoustic Emissions. Otoacoustic emissions were evoked by rarefaction clicks generated by 0.1-ms rectangular pulses delivered to the earspeaker. Stimulus level was controlled by a calibrated attenuator. The stimuli were delivered at a repetition rate of 19/s. The spectrum of the acoustic click as recorded with the microphone-coupler system was nearly flat within ±5 dB from 0.5 to 3 kHz. For clinical use, the level of the stimulus was expressed in decibels hearing level (dB HL) (ie, relative to the normal subjective threshold for the sequence of clicks measured in ten normally hearing

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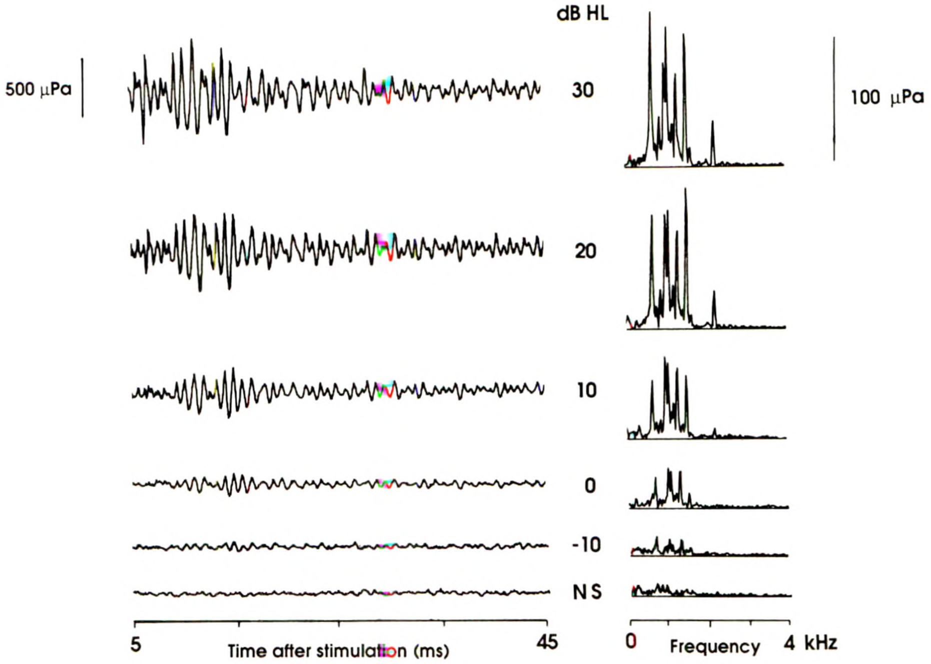

Fig 1. Typical example of EAEs recorded from normally hearing subject. EAEs were recorded at decreasing click intensities ranging from 30 to −10 dB HL. Subjective threshold for sequence of clicks was 0 dB HL. NS — no stimulus condition. Left) Time domain averages (n = 1,024) for clicks. Signal was analyzed in 40-ms window starting 5 ms following stimulus onset. Duration of EAEs was more than 20 ms. Peak-to-peak amplitude of EAEs increased with increasing click level, but saturation appeared at 30 dB HL. Threshold of EAEs was estimated at −10 dB HL. Right) Spectral analyses of averaged ear canal signals (linear vertical scale). Spectra show several narrowband peaks superimposed on broadband component.

subjects).

Evoked acoustic emissions were recorded with the subject comfortably reclining in a sound isolation chamber. The output of the microphone was fed into an amplifier (Medelec AA6 Mk3) operated at gains of 1,000 to 10,000. After amplification, responses were band pass filtered (high pass: 250 Hz, 16 dB per octave; low pass: 8 kHz, 6 dB per octave), sampled over 1,024 points within a time window of 30 to 40 ms starting 5 ms after stimulus onset and averaged in the time domain over 512 to 2,048 repetitions. An artifact rejection system was used to reject any epoch contaminated by artifacts. All results were stored on flexible disks for later analysis. With this method, the noise floor of the measuring system was reduced to about −5 dB sound pressure level (SPL) in the frequency range of 0.5 to 5 kHz.

The averaged ear canal signal was subsequently fed into a high resolution signal analyzer (Hewlett Packard 3661 A) for fast Fourier transform (FFT) analysis. Fast Fourier transform of the time-averaged acoustic signal was done over a frequency span of 10 kHz and a frequency resolution of 37.5 Hz. Spectra were displayed on a linear scale. Evoked acoustic emissions were recorded at decreasing stimulus intensities ranging from 30 to 40 dB HL to detection threshold in 10-dB steps (5 dB near threshold).

Data Analysis. Identification of EAEs was based upon several criteria: 1) nonlinear saturating responses to higher stimulus levels,[2,5] 2) discrete frequency-specific maxima in the FFT analyses,[3] and 3) repeatability of the EAE waveform.[2,14] The following parameters were analyzed: duration, detection threshold, and frequency content. On the basis of EAE duration, ears were classified into short (< 20 ms after the onset of averaging) and long (> 20 ms) EAEs, as earlier proposed by Zwicker[4] and Probst et al.[6] Thresholds of EAEs were determined using visual detection threshold criteria in both time and frequency domains. Spectral peaks were identified from FFTs of averaged waveforms and the frequency maxima were measured. Statistical analyses of the data were performed using Student's *t* test with a level of significance set to a probability of .05 or less.

Subjects. Two groups were studied. The first was a control group (n = 52 ears) including normally hearing young adults without any history of otologic disease or exposure to ototoxic agents or noise and with normal otologic findings (normal otoscopic findings, normal middle ear pressure, and normal stapedius reflex thresholds). All subjects had audiometric pure tone thresholds of 10 dB HL or less at octave frequencies between 0.25 and 8 kHz. Ages ranged from 14 to 49 years (mean age, 23.4 years). The second group (n = 85 ears) consisted of patients exhibiting a pure sensorineural hearing loss (based upon audiogram, acoustic reflex, and auditory brain stem responses). Ages ranged between 19 and 76 years (mean, 39.3 years). Sensorineural hearing losses were of various causes: viral infection, noise, ototoxic drugs, presbycusis, or heredity. The majority of the ears exhibited dominant high-tone hearing losses. Patients with Meniere's disease and acoustic neuroma were excluded because EAEs showed specific features that will be analyzed in forthcoming papers.

RESULTS

Control Group (n = 52). Click stimuli elicited EAEs from 100% of the ears of the normal population. Figure 1 shows a typical example of EAEs

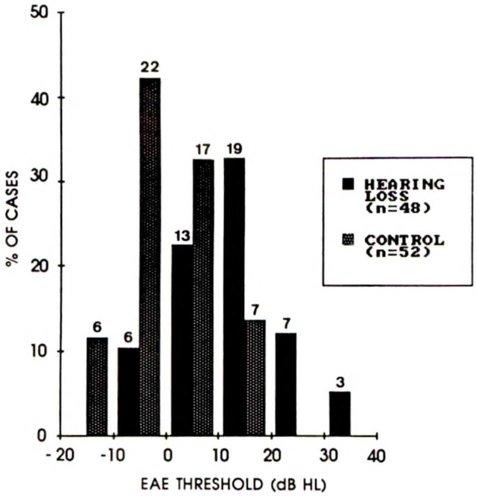

Fig 2. Histogram of EAE detection thresholds. Number of patients taken into account for each column is indicated at top of that column. Means and standard deviations for both populations are indicated in Table.

from a normal ear. We were unable to record EAEs at intensities above 40 dB HL because the otoacoustic response was contaminated by a large acoustic artifact that made the identification of EAEs difficult. Across ears, EAEs differed in duration, detection threshold, and frequency spectrum. Seventeen ears (33%) demonstrated short duration (<20 ms) EAEs, and 35 (67%) long lasting (>20 ms) EAEs. Figure 2 shows the distribution of EAE detection thresholds in the control group. Evoked acoustic emissions threshold was less than the normal hearing level (0 dB HL) in 54% of the ears. The mean EAE detection threshold was −5.2±2.4 dB HL.

Two distinct patterns of EAEs were identified with respect to spectra. All EAEs exhibited a broadband spectrum with a frequency maximum near 1.2 kHz. Fifty-seven percent of EAEs demonstrated several narrowband frequency peaks superimposed on the broadband component. The number of spectral peaks evoked by 30-dB clicks ranged from one to seven with an average of three per ear. Narrowband peaks were only observed in long-duration EAEs. Short duration EAEs were generally broadly tuned and devoid of narrowband peaks (see Table).

Cochlear Hearing-Impaired Group ($n = 85$). The clicks evoked emissions in 48 ears (56%) with sensorineural hearing loss. The incidence of EAEs decreased linearly with increasing audiometric threshold (Fig 3). Evoked acoustic emissions were not obtained from ears with subjective click threshold shifts of 35 dB HL and above. Evoked acoustic emissions rarely were observed when the mean audiometric threshold for frequencies of 2 and 4 kHz exceeded 35 dB HL. However, clear EAEs could be recorded in a few ears with significant high frequency hearing losses (>35 dB) exhibiting some preservation of hearing at 1 kHz and below (Fig 4). The Table summarizes the principal characteristics of EAEs in normal and cochlear loss groups. Hearing-impaired ears showed a considerably higher proportion of short duration EAEs than did normal ears ($p < .001$, see Table).

Figure 2 shows the distribution of EAE thresholds in the cochlear hearing loss group along with the control group. Hearing-impaired ears revealed significantly elevated EAE detection thresholds compared to those of controls (see Table, $p < .001$). Sixty percent of ears from the cochlear hearing-impaired group exhibited EAE thresholds above 10 dB HL. Evoked acoustic emissions detection threshold increased linearly with increasing subjective click threshold (Fig 3).

In the cochlear hearing-impaired group a broadband response was always present in the 0.7- to 1.5-kHz band, with a mean maximum response energy occurring at 1.1 kHz. However, hearing-impaired ears showed a considerably reduced proportion of narrowband peaks when compared with normal ears ($p < .001$, see Table). The incidence of narrowband peaks decreased from 57.5% in normal ears to 10% in ears with a subjective click threshold of 20 dB HL to a drop to 0% in ears with a subjective click threshold of 30 dB HL. Moreover, the mean number of narrowband peaks per ear was significantly reduced in patients with a sensorineural hearing loss ($p < .01$, see Table).

MAIN PROPERTIES OF EVOKED ACOUSTIC EMISSIONS IN NORMAL EARS AND EARS WITH SENSORINEURAL HEARING LOSS

	Control Group ($n = 52$)	*Hearing Loss Group ($n = 85$)*	*Significant Difference*
Incidence of EAEs	100.0%	56%	p<.001
Duration of EAEs			
Short (<20 ms)	33.0%	85%	p<.001
Long (>20 ms)	67.0%	15%	p<.001
Detection threshold in dB HL (mean ± SD)	−5.2±8.6	7.5±13.8	p<.001
Spectral analysis			
Broadband spectral component alone	42.5%	82%	p<.001
Broadband spectral component + narrowband peaks	57.5%	18%	p<.001
Peak of maximum energy of broadband compound in Hz (mean ± SD)	1,210±404	1,160±236	NS
Mean number of narrowband peaks per ear	3	1.7	p<.01

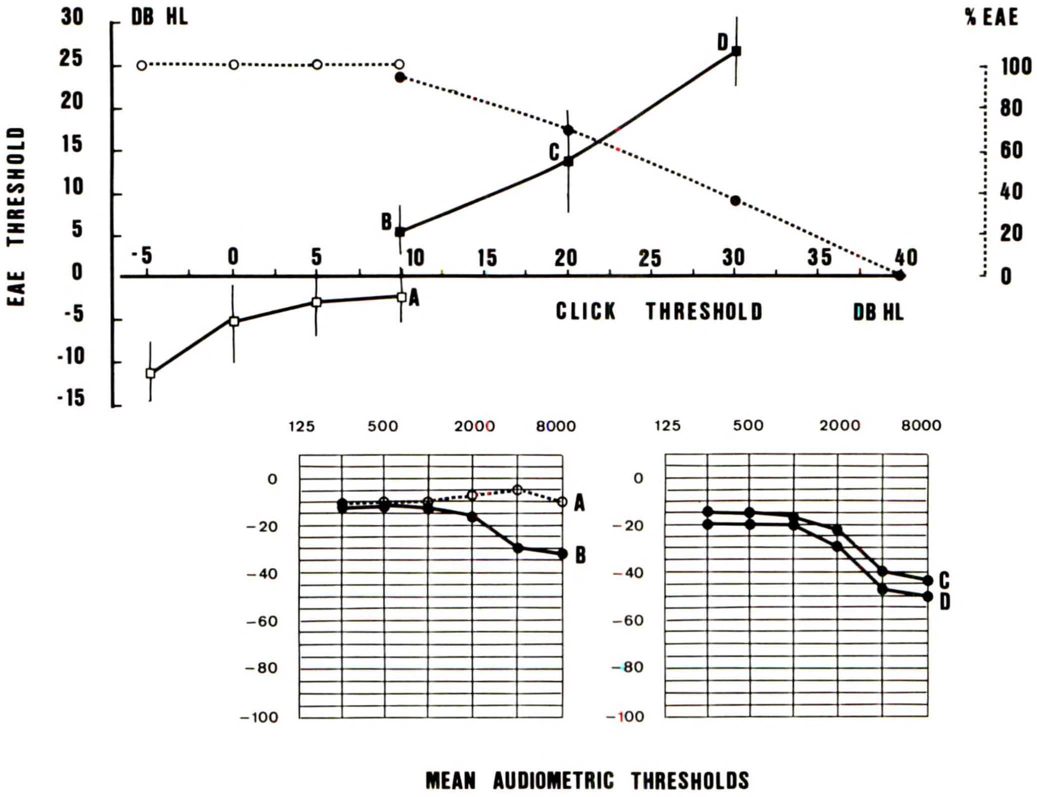

Fig 3. Evolution of mean EAE threshold (□■, solid lines) and incidence of EAEs (○ ●, dotted lines) as function of subjective click threshold (in dB HL) in normally hearing ears (open symbols) and ears with sensorineural hearing loss (black symbols). Ears were divided in eight subgroups exhibiting subjective click thresholds respectively at −5, 0, 5, and 10 dB HL for normal ears, and 10, 20, 30, and 40 dB HL for hearing-impaired ears. Mean audiograms of four of sets of patients are shown. A — group of normally hearing patients with click threshold at 10 dB HL and no high tone hearing loss; B, C, D — groups of hearing-impaired patients with click thresholds respectively at 10, 20, and 30 dB HL and high tone hearing loss. In control group (○--), EAEs were present in all ears (incidence = 100%). Mean EAE thresholds (□—) increased with increasing subjective click thresholds with saturation at subjective click threshold of 5 dB. However, mean EAE thresholds were less than 0 dB HL. In sensorineural hearing-impaired group, incidence of EAEs (● --) decreased and mean EAE threshold (■—) increased with increasing subjective click threshold. No EAEs were evoked from ears exhibiting subjective click threshold of 40 dB HL and above. Vertical bars indicate standard error.

DISCUSSION

This paper shows that 1) EAEs can be detected in all normal ears, 2) the incidence of EAEs decreases and the detection threshold of EAEs increases with sensorineural hearing loss, and 3) EAEs are not observed in ears exhibiting threshold shifts of 30 to 35 dB HL. These results are consistent with the use of EAEs as a simple, objective, clinical test for screening cochlear status.

It is not clear from the literature whether EAEs can be detected in 100% of normal ears. In his initial report, Kemp[2] was able to record emissions from all of the 37 ears he examined. This observation was confirmed by other investigators.[16,17] However, several authors have reported a 90% incidence of EAEs in normal ears,[19,20] and others even smaller incidences.[4,21] Probst et al[6] clearly demonstrated that stimulus parameters may influence the incidence of EAEs: 0.5-kHz tone bursts evoked emissions in 36% of ears, 1.5-kHz tone bursts in 100%, and clicks in 96%. Therefore clicks seem to be a good stimulus for use in clinical diagnostic testing, since they are able to elicit emissions in nearly all normal ears.

Existing data on EAEs in hearing-impaired ears also show discrepancies in the relationship between the amount of hearing loss and the presence of EAEs. In his initial description of EAEs in 27 ears with various degrees of hearing loss, Kemp[2] noted that EAEs, in general, were not observed from ears in which threshold shifts as small as 30 dB HL had occurred. In a later study on eight patients, Rutten[19] reported that the presence of EAEs in response to tone bursts has limited applicability as an objective measure of hearing dysfunction since EAEs could not be detected if the hearing loss at the tone burst frequency exceeded 15 dB. In contrast, Hinz and von Wedel[22] detected EAEs in ears with hearing loss greater than 30 dB due to sudden deafness. Our study confirms that the incidence of EAEs decreases linearly with increasing audiometric threshold. It is consistent with the recent study by Probst

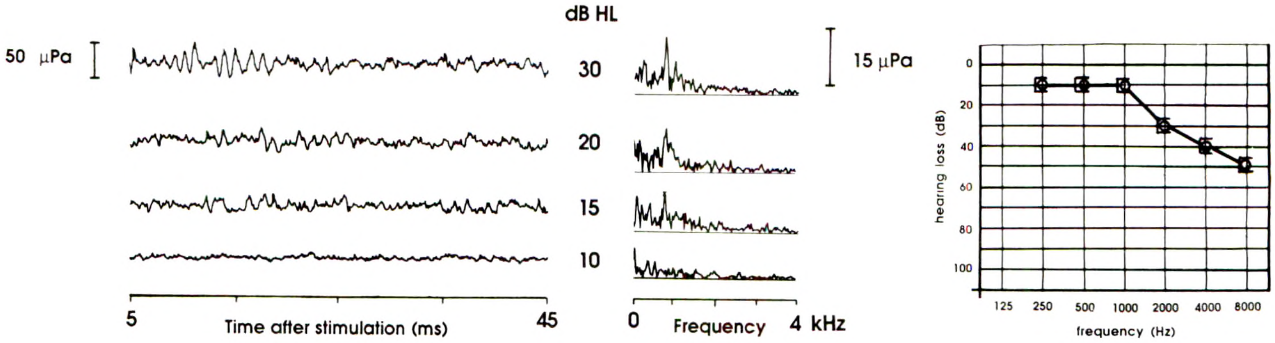

Fig 4. Typical example of EAEs in ear with pure sensorineural hearing loss. Audiogram shows high frequency hearing loss with preservation of hearing at frequencies below 1 kHz. ○ — air conduction threshold. [— bone conduction threshold. Subjective threshold for sequence of clicks was 15 dB HL. Ear demonstrated short duration EAEs with one broad spectral peak at 800 Hz. Threshold of EAEs was estimated at + 15 dB HL. Note considerable reduction in EAE amplitude when compared to normally hearing ear (Fig 1).

et al[18] on 44 hearing-impaired ears that showed that EAEs were detected in 34 of 35 ears with a subjective click threshold < 25 dB HL but not in nine ears with a subjective click threshold > 25 dB HL. However, our study (Fig 4) as well as the study by Probst et al[18] showed a few cases in which EAEs could be recorded in ears that exhibited a significant high frequency hearing loss (> 30 dB HL) with relative preservation of hearing at low frequencies and middle frequencies. The incidence of EAEs may also depend on the intensity of the stimulation since we did not use stimuli above 40 dB HL. Actually, it is likely that the incidence of EAEs in hearing-impaired ears would not be changed by higher levels of stimulation because the response energy of ears with thresholds above 30 dB HL is reduced to levels near that of noise,[17] and then the low acoustic response would be obscured by a large acoustic artifact. Further experiments are required to study the influence of parameters such as the shape of the audiogram and the cause of the hearing loss on EAE properties, which may also account for the discrepancies discussed above.

The detection threshold of EAEs seems to be the main parameter to be used for clinical applications. This study confirms the observations previously reported by several investigators[2,14,16] that in normal ears EAE threshold is generally lower than the subjective threshold by about 10 dB. In hearing-impaired ears, EAE threshold increases almost linearly with increasing subjective click threshold. Using a different procedure, Kemp et al[17] showed a gradual decrease in acoustic emission level with sensory hearing loss with roughly a 10-dB loss in emission level for a 10-dB loss of hearing.

Other parameters such as latency, duration, and frequency spectrum seem to be of little interest for clinical applications. Latency of EAEs is difficult to measure with accuracy because of the stimulus artifact. Hearing-impaired ears show a higher incidence of short duration EAEs than do control ears. However, this parameter is of poor predictive value since short duration EAEs can also be observed in 20% of normal ears[6] (33% in the present study). Both hearing-impaired and normally hearing ears appear to emit similar patterns of spectral peaks.[18] The only difference is that narrowband spectral peaks are less often encountered in hearing-impaired ears.

There is strong evidence that the mechanism responsible for EAEs is located in the cochlea, as reviewed by Wilson.[23] Emissions have been shown to be reduced or eliminated by several factors known to influence cochlear function such as anoxia, treatment with ototoxic drugs, or noise exposure, as reviewed by Wilson.[23] The exact origin of EAEs is yet unknown, but a number of recent findings suggest that outer hair cells (OHCs) could be involved in EAE production, as reviewed by Pickles.[24] Evoked acoustic emissions probably reflect the active biomechanical processes within the cochlea.[24] These processes are involved in producing the sharp tuning of the traveling wave, of inner hair cells, and of auditory nerve fibers, and probably arise in OHCs, which exhibit motile properties in vitro.[25,26] The most recent hypotheses suggest that OHCs give the inner hair cells an amplified and sharply tuned mechanical stimulation.[24] Evoked acoustic emissions thus could be used as an indicator of the biomechanical status of the cochlea. This test could provide indirect information on OHC function, which accounts for the exquisite properties of sensitivity and frequency selectivity of the cochlea. It is not surprising that EAEs are altered whenever a mild loss of hearing occurs because OHCs are known to be damaged first in all diseases involving the cochlea.[27-29] In this way, EAEs provide complementary information on cochlear function that is not available from conventional electrophysiologic evoked responses (auditory brain stem response [ABR] and electrocochleography).

This study shows that EAEs can be used as a reliable technique for studying cochlear function. First, it is a simple and noninvasive test. Recordings only require the usual ABR recording system with an additional sound probe and an FFT analyzer. Second, it is a rapid and objective test that allows for a quick determination of a threshold that is quite near the audiometric threshold, and that may provide an objective assessment of the functional condition of the micromechanical mechanisms of the cochlea. A single recording of EAEs with a click level of 10 dB HL can be achieved in 2 minutes and can indicate a normal cochlear function. Elevated EAE threshold suggests a mild sensorineural hearing loss. On the contrary, the absence of EAEs suggests a moderate-to-profound hearing loss. However, it is very important to assess the integrity of the middle ear by otoscopy and tympanometry before recording EAEs because middle ear disease may alter EAE production greatly.[17] At the present time, EAEs have not yet yielded information useful in differentiating distinct causes of hearing loss.[17,18]

Future clinical applications of EAE recordings could be envisaged.

1. Early detection of hearing loss in infants and children could be accomplished using the EAE analysis of a low level of stimulation.
2. The supervision of a high risk population (ototoxicity, noise trauma) by EAE recordings could be more sensitive than other measures because these hearing losses first involve OHCs.[27-29]
3. The recording of EAEs could provide complementary data in favor of a retrocochlear hearing loss in acoustic neuromas, especially in the case in which ABR does not exhibit typical abnormalities.
4. Since EAEs provide information on the mechanical properties of the cochlea, they could be of major interest in the assessment of Meniere's disease and could be used as an easy and objective investigation along with the osmotic test.

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RECURRENT TONSILLITIS: HISTOLOGIC AND BACTERIOLOGIC EVALUATION

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Histologic and bacteriologic evaluations of tonsils removed at surgery from ten patients with a diagnosis of recurrent tonsillitis were performed. The bacteriology was complex, with an average of 6.3 aerobic bacteria and 3.3 anaerobic bacteria isolated from each patient. Histologic sections revealed chronic cryptitis, with intact tonsillar architecture. These findings provide a possible explanation for the failure of commonly used antibiotic regimens to eradicate recurrent infection from this site.

KEY WORDS — bacteria, histology, tonsillitis.

INTRODUCTION

Acute tonsillitis, caused by *Streptococcus pyogenes* (group A β-hemolytic *Streptococcus*), is frequently encountered in children and young adults. The use of penicillin for this infection is effective because of its excellent activity against the causative organism. A subset of patients with acute tonsillitis will develop recurrent tonsillitis despite penicillin therapy. This recurrent symptomatic tonsillitis often requires surgery for cure. Despite the frequent occurrence of this disease, the causative agent(s) of and pathologic basis for this infection have not been well studied.

Previous investigators have found a mixed aerobic-anaerobic bacterial flora present in recurrently inflamed tonsils. The methods of obtaining material for culture varied in these reports.[1-3] A study was undertaken at Tufts–New England Medical Center to define the causative agents and histologic changes encountered in this infection. We determined that understanding the pathologic basis of recurrent tonsillitis might encourage future investigations of other antimicrobial agents for its treatment.

MATERIALS AND METHODS

Bacteriologic cultures were obtained from one tonsil from each of ten patients undergoing bilateral tonsillectomy for recurrent tonsillitis. The patients ranged from 5 to 32 years of age, with a mean age of 14.2 years. The decision for tonsillectomy had been made previously by a member of the Department of Otolaryngology. The presence or absence of prior streptococcal infection was not used as a criterion for surgery. The clinical characteristics of these patients are summarized in Table 1. The patient population was felt to reflect such an unselected group as might be encountered in the office practice of otolaryngology. Precise data concerning the number of episodes of tonsillitis, the time span over which recurrences occurred, and the number of courses of antibiotic therapy used could not be ascertained from these patients or their relatives. One patient was taking penicillin at the time of admission for tonsillectomy, but the remaining patients had not received antibiotics for at least 1 week prior to admission.

In our opinion, it is impossible to differentiate chronic from recurrent tonsillitis, and we suggest that these two terms be used to represent the same disease process. Only if a patient's tonsils returned grossly and histologically to normal between episodes could recurrent tonsillitis be differentiated from chronic tonsillitis.

After surgical removal, the tonsils were transported immediately from the operating room to the laboratory, where one tonsil was sectioned in half and a small piece of tissue was removed from the middle, ground with a mortar and pestle, and cultured as soon as possible. Care was taken to avoid surface contamination by using a heated scalpel to make the surface incision. The tissue was plated on trypticase soy agar with 5% sheep blood, MacConkey, and chocolate agar plates for identification of aerobic and facultative organisms. These cultures were incubated at 37°C in carbon dioxide and examined after 18 hours. The tissue was plated similarly on 5% sheep blood with brucella base (supplemented with hemin, vitamin K, and yeast extract), laked kanamycin-vancomycin, and phe-

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From the Department of Medicine (Infectious Diseases) (Bieluch, Tally), the Department of Pathology (Martin), the Department of Otolaryngology (Chasin), and the Department of Pathology, Clinical Microbiology Laboratory (Tally), New England Medical Center Hospitals, Boston. Dr Tally is a recipient of Research Career Development Award AI 00393 from the National Institutes of Health/National Institute of Allergy and Infectious Diseases. The study was also supported by a grant from the Upjohn Company.

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TABLE 1. CLINICAL CRITERIA OF PATIENTS UNDERGOING TONSILLECTOMY

Patient	*Surgeon's Preoperative Diagnosis*	*Episodes of β-Hemolytic Streptococcal Infection*	*Indication for Tonsillectomy*	*Age at Surgery (yr)*	Hemophilus *Species Present*
1	Chronic tonsillitis	6	Recurrent throat infections for 5 yr causing 40 d missed in one school year	12	Yes
2	Recurrent tonsillitis	10-12	Recurrent, severe throat infections	9	No
3	Recurrent tonsillitis	"Several"	Recurrent tonsillitis for 3 to 4 yr	7	Yes
4	Recurrent tonsillitis	0	Recurrent tonsillitis	23	Yes
5	Recurrent tonsillitis	1	Recurrent sore throat for 13 yr	25	No
6	Recurrent tonsillitis	1	Recurrent sore throat	5	No
7	Chronic tonsillitis	0	Chronic sore throat	19	Yes
8	Recurrent tonsillitis and hypertrophied T & A	>1	Hypertrophic tonsils causing obstruction to swallowing and breathing	5	Yes
9	Recurrent tonsillitis	5 times in past year	Recurrent tonsillitis	32	No
10	Recurrent tonsillitis	Occasional positive culture for *Streptococcus*	Recurrent throat infections	5	Yes

nylethyl alcohol agar plates for selection of anaerobic bacteria. These plates were incubated in an anaerobic chamber at 37°C and examined after 48 hours. Additional tissue was inoculated into chopped meat broth. Identification of aerobic bacteria was performed by standard methods, and identification of anaerobic bacteria was performed according to the Virginia Polytechnic Institute recommendations (L. V. Holdeman et al, unpublished data).

Histologic sections of right and left tonsils from each patient were reviewed. These sections of tonsillar tissue were stained with hematoxylin and eosin, kinyoun, Gram's, and methenamine-silver stains, after fixation with 10% neutral-buffered formalin.

RESULTS

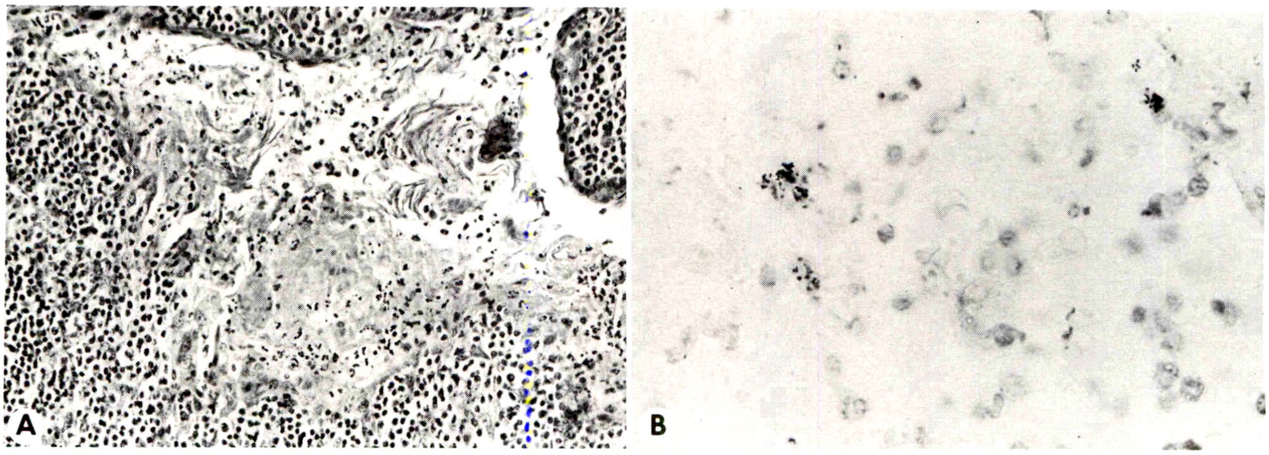
The bacterial flora from the core of recurrently inflamed tonsils from these ten patients is complex, involving a number of aerobic and anaerobic pathogens (Table 2). The average number of aerobic bacteria isolated was 6.3, with a range of three to nine organisms isolated per patient. The average number of anaerobic bacteria isolated was 3.3, with a range of one to eight per patient. The most frequently isolated potential aerobic pathogens belong to the *Hemophilus* group. These organisms were isolated from six of ten patients and were found in both adults and children (one patient had two isolates, one *Hemophilus influenzae* and one *Hemophilus* species) (Table 2). In addition, there were four patients with *Eikenella corrodens* and four with *Staphylococcus aureus*. Group A β-hemolytic streptococci, the most commonly identified cause of acute tonsillitis, were isolated from only one patient.

Almost all the patients had anaerobic gram-positive cocci isolated from tonsillar tissue. Seven of ten patients had *Bacteroides* species. Fusobacteria were isolated from four patients, although only one patient had *Fusobacterium nucleatum*. There was a single patient who harbored a possible *Actinomyces israelii*, and a second who was infected with *Actinomyces odontolyticus*.

Histologic evaluation revealed various degrees of lymphoid hyperplasia in these tonsils. The underlying tonsillar architecture remained intact, however.

TABLE 2. AEROBIC AND ANAEROBIC BACTERIAL ISOLATES IN CHRONICALLY INFECTED TONSILS

Organism	*No. of Patients*
Aerobic	
Staphylococcus aureus	4
Staphylococcus sp	1
α-Hemolytic *Streptococcus*	10
β-Hemolytic *Streptococcus*, group A	1
β-Hemolytic *Streptococcus*, non–group A	1
γ-Hemolytic *Streptococcus*	2
Streptococcus pneumoniae	1
Actinomyces odontolyticus	1
Possible *Actinomyces* sp	1
Diphtheroids	1
Neisseria sp	9
Eikenella corrodens	4
Hemophilus influenzae	5
Hemophilus sp	2
Micrococcus sp	5
Capnocytophaga sp	2
Acinetobacter anitratus	1
Gram-variable cocci	1
Gram-negative rods, nonenteric	2
Gram-positive rods, not *Actinomyces*	3
Anaerobic	
Gram-positive cocci	8
Aerotolerant streptococci	1
Gram-positive, non–spore-forming rods	2
Actinomyces odontolyticus	1
Possible *Actinomyces israelii*	1
Possible *Actinomyces* sp	1
Veillonella sp	2
Bacteroides melaninogenicus	4
Bacteroides sp	3
Fusobacterium sp	4
Gram-negative cocci	1



Histologic evaluation of tonsillar crypts. A) Low power view of cellular and keratinous debris shows acute inflammatory cells (H & E, original x100). Focal destruction of crypt epithelium is demonstrated. B) Tonsillar tissue shows gram-positive organisms (Gram's stain, original x630).

In some cases there was chronic inflammation in the peritonsillar tissues, including the minor salivary glands and skeletal muscle. Acute inflammatory cells were limited to the crypts, where collections of keratinous debris and polymorphonuclear leukocytes were seen in the lumen in every case (see Figure, A). The squamous epithelium lining the crypts occasionally contained acute inflammatory cells, while in other cases, there was ulceration of the crypt lining.

Sulfur granules were visualized in tonsils from two of our ten patients by means of hematoxylin and eosin stain. Gram's stain revealed gram-positive cocci of various sizes in the tonsillar crypts in nine of the ten patients studied (see Figure, B). Occasional gram-positive and gram-negative bacillary forms were also noted. Organisms were not detected in the tonsillar parenchyma. No acid-fast organisms were identified. The methenamine-silver stain revealed the presence of *Actinomyces* in the two patients in whom sulfur granules were seen with the hematoxylin and eosin stain. No fungi were seen. In one patient, no organisms were visualized with any of the staining techniques.

DISCUSSION

Previous investigators have implicated anaerobic bacteria as causative agents in recurrent tonsillitis.[1-3] Our study of ten patients, both children and adults, has confirmed the bacteriologic findings of others, namely that the flora of these tonsils is complex and comprises both aerobic and anaerobic bacteria. These organisms are the ones that are normally found in the oropharyngeal cavity. Multiple potentially pathogenic organisms were cultured from each patient, and it is impossible to know which are the pathogens responsible for the infection. However, it may well be that the presence of more than one species of bacteria is necessary for infection to occur.

Of the aerobic bacteria, members of the *Hemophilus* group seem to be important as potential pathogens, as they were isolated from seven of the ten patients. In contrast to the findings in acute tonsillitis, group A β-hemolytic streptococci were found in only one patient. The predominant anaerobic bacteria isolated were *Bacteroides* species, isolated from seven of the ten patients.

Cultures for other organisms such as viruses, mycoplasma, and chlamydia were not performed as part of this study. Thus, no conclusions can be made concerning the potential causative role of these organisms.

Histologic examination confirmed the previously described findings of an exuberant cryptitis present in the tonsils, without infiltration of tonsillar tissue by the inflammatory cells.[4] The histology was uniform, despite the fact that there was variability in the length of illness of the patients. The fact that these tonsils were histologically abnormal at a time when patients were clinically asymptomatic suggests that differentiation of "recurrent" and "chronic" tonsillitis appears to be difficult.

Penicillin, because of its activity against group A β-hemolytic *Streptococcus*, has been the mainstay of therapy for acute episodes of pharyngitis caused by this organism. Furthermore, because of its activity against group A β-hemolytic *Streptococcus*, as well as its activity against most of the bacteria normally inhabiting the oropharynx, this antibiotic has been used by physicians empirically for the treatment of pharyngitis when no attempt is made to identify a pathogen by culture. As histologic studies indicate, the problem appears to be bacterial infection of the tonsillar crypts, rather than of the tonsils themselves. Because of the low minimum inhibitory concentrations of penicillin required to destroy most of the organisms that normally inhabit the oral cavity, even low levels of penicillin in the tonsillar crypts would eradicate these organisms effectively. Organisms resistant to penicillin thus would be left to inhabit this ecologic niche. Thus, the complex bacterial flora present in chronic tonsillitis may reflect the selection of penicillin-resistant organisms from the oropharyngeal flora and subsequent chronic cryptitis due to these organisms. Achieving adequate antibiotic concentrations to kill relatively resistant organisms in these crypts, the site of inflammation, will be difficult because of diffusion of antibiotic out of the crypts into the oropharyngeal cavity.

Presently, surgery seems the only established mode of therapy for tonsil infections that continue to recur after multiple courses of antibiotics. This fact could well be explained by the histologic findings of cryptitis demonstrated in this study. Recently, a group of investigators has shown that surgical treatment of children severely infected with recurrent throat infection results in fewer recurrent infections than does medical therapy, although this difference begins to disappear after 2 to 3 years of follow-up.[5] During those initial years, however, a price must be paid in terms of school days, parental work days, doctor visits, antibiotic cost, and adverse effects. The findings of this study, namely that complex bacterial flora and cryptitis exist with the remaining tonsillar architecture intact, suggest that one avenue of future research might be evaluation of longer-term antibiotic therapy with a broader spectrum oral antibiotic or antibiotic combination as treatment for recurrent tonsillitis. It would be helpful to have a therapeutic alternative to tonsillectomy, given the potential for complications as a result of this operation.

ACKNOWLEDGMENT — We thank Susan W. Cooper, MS, for performing the bacterial analysis.

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THE DEAFNESS RESEARCH FOUNDATION
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The Deafness Research Foundation invites applications for 1990 grant support of new research that promises to advance our understanding of the causes, treatment, and prevention of deafness and related ear disorders. The grants will be awarded to tax-exempt institutions for projects that will begin January 1, 1990, with the principal investigator of each project a member of the staff or faculty of such institution. The grants do not exceed $15,000, and can be competitively renewed with the same limit for an additional 1 or 2 years. Applications are reviewed for scientific merit of the proposed investigations and for their direct or potential clinical importance. The deadline for first year applications is July 15, 1989, and for renewal applications is August 15, 1989. For full information on the grant program, and for the required application form, please write to Walter A. Petryshyn, MD, Medical Director, The Deafness Research Foundation, 9 East 38th Street, New York, NY 10016; (212) 684-6556.

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DIMENSIONS OF THE EXTENDED FACIAL RECESS

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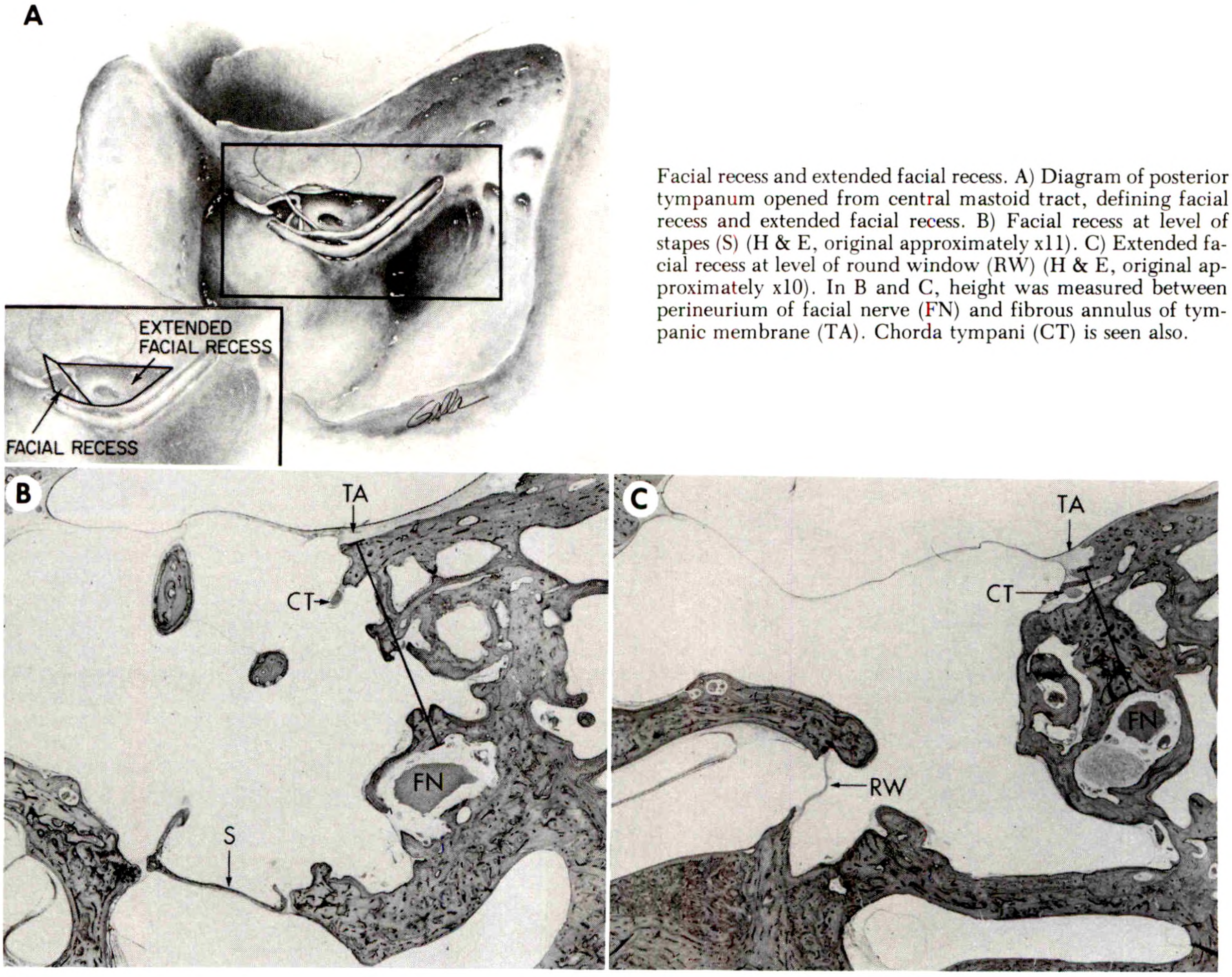
The variability of the dimensions of the facial recess and extended facial recess with age, sex, and degree of pneumatization was measured in 87 temporal bones serially sectioned in the horizontal plane. Although some statistically significant differences in dimensions were identified, the absolute differences were small compared to the range of measurements. The mean dimensions were very similar in all groups studied. The clinical relevance of the data to mastoid surgery and cochlear implantation is discussed.

KEY WORDS — extended facial recess, facial recess, temporal bone.

INTRODUCTION

Surgical access from the central mastoid tract to the posterior tympanum, or the "facial recess approach," commonly is used in canal wall up mastoidectomy for chronic ear disease or as a means of access to the round window niche for implantation of intracochlear electrodes in the profoundly deaf. Variation in the dimensions of the pneumatized spaces of the temporal bone, as determined by the degree of pneumatization and growth and development, has been described.[1] However, there is a paucity of information concerning the dimensions of the "facial recess" and how these dimensions vary with age, sex, and degree of pneumatization.



Facial recess and extended facial recess. A) Diagram of posterior tympanum opened from central mastoid tract, defining facial recess and extended facial recess. B) Facial recess at level of stapes (S) (H & E, original approximately x11). C) Extended facial recess at level of round window (RW) (H & E, original approximately x10). In B and C, height was measured between perineurium of facial nerve (FN) and fibrous annulus of tympanic membrane (TA). Chorda tympani (CT) is seen also.

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TABLE 1. HEIGHT OF FACIAL RECESS IN MILLIMETERS

		At Stapes			*At Round Window*		
Variable	*n*	*Mean*	*SE*	*Range*	*Mean*	*SE*	*Range*
Ear							
Left	41	3.85	.106	2.5-5.4	2.95	.082	1.8-3.8
Right	46	3.97	.064	2.9-5.0	3.00	.066	1.6-4.2
Sex							
Male	51	4.02	.078	2.8-5.4	3.01	.078	1.6-4.2
Female	36	3.78	.091	2.5-4.8	2.93	.060	2.1-3.4
Sex (adults only)							
Male	27	4.07	.110	2.8-5.4	3.11	.082	2.3-4.2
Female	20	3.83	.138	2.5-4.8	2.88	.069	2.2-3.3
Age							
Adult	47	3.97	.087	2.5-5.4	3.01	.058	2.2-4.2
Child	40	3.86	.082	2.7-5.0	2.93	.091	1.6-3.9
Pneumatization (adults only)							
Good	36	4.00	.094	2.5-5.5	3.00	.066	2.3-4.2
Poor	11	3.87	.220	2.8-4.9	3.05	.125	2.0-3.6
Pneumatization (adults only, adjusting for sex)							
Male							
Good	19	4.20	.114	3.3-5.4	3.16	.093	2.3-4.2
Poor	8	3.77	.231	2.8-4.9	2.99	.170	2.0-3.6
Female							
Good	17	3.77	.134	2.5-4.7	2.83	.074	2.2-3.3
Poor	3	4.15	.578	3.0-4.7	3.19	.058	3.1-3.3

SE — standard error.

TABLE 2. SUMMARY OF ANALYSES OF VARIANCE IN 60 EARS IN 60 INDIVIDUALS (36 ADULTS, 24 CHILDREN)

Variable	*Sum of Squares*	*Degrees of Freedom*	*F-Value*	*p*
Outcome, mean facial recess at stapes				
Sex	0.844	1	2.50	.12
Pneumatization	0.238	1	0.71	.40
Interaction	0.004	1	0.01	.92
Error	18.904	56		
Outcome, mean facial recess at round window				
Sex	0.003	1	0.01	.91
Pneumatization	0.025	1	0.10	.76
Interaction	0.768	1	2.92	.09
Error	14.709	56		

The nomenclature used to define the space between the descending facial nerve and the tympanic annulus and chorda tympani deserves comment. "Facial recess" is a surgical rather than anatomic term referring to the potential space bounded superiorly by the fossa incudis, medially by the descending segment of the facial nerve, laterally by the tympanic annulus, and inferiorly by the chorda tympani nerve.[2,3] This space is created by opening a pneumatized extension of the posterior tympanum, called the "sinus posterior," from a mastoid approach.[3] For greater access to the posterior tympanum and hypotympanum, the chorda tympani may be sacrificed, allowing a bony opening to be created between the tympanic annulus and the descending segment of the facial nerve both superior and inferior to the course of the chorda tympani, which will be called here "the extended facial recess" but has been shortened by some authors to simply "the facial recess"[4] (see Figure, A). This study was designed to determine the height of the extended facial recess, that is, the distance between the facial nerve and tympanic annulus, and its variability with age, sex, and degree of pneumatization of the temporal bone.

MATERIALS AND METHODS

Eighty-seven temporal bones from the collection of the Massachusetts Eye and Ear Infirmary were selected for study. They included 47 temporal bones from adults and 40 temporal bones from children from birth up to 9 years of age. The adult temporal bones were graded on the basis of degree of pneumatization. "Poor" pneumatization implied pneumatization of the antrum but limited pneumatization of the central mastoid tract and no pneumatization of the perilabyrinthine tract, petrous apex, or accessory regions. A well-pneumatized central mastoid tract with at least some pneumatization of the perilabyrinthine tract, petrous apex, and accessory regions was judged as having "good" pneumatization.

The temporal bones were sectioned in the horizontal plane, that is, roughly parallel to the floor of the middle fossa at a thickness of 20 μm. Every tenth section was stained with hematoxylin and eosin. The sections were examined with a Bausch and Lomb microprojector. The height of the facial recess and extended facial recess, defined as the medial to lateral distance between the perineurium of the descending segment of the facial nerve and the fibrous tympanic annulus (see Figure; B,C), was measured along two segments of its length: 1) in all sections in which the stapes footplate was present and 2) in all sections in which the round window membrane was present. The mean of the measurements of height taken in sections in which the stapes footplate was present was called "the mean height of the extended facial recess at the stapes," and the mean of the measurements taken from sections in which the round window membrane was present was called "the mean height of the extended facial recess at the round window." Statistical significance of differences was evaluated by the two-way analysis of variance (ANOVA) and the two-tailed Student's t test, with a $p = .05$ criterion of significance unless stated otherwise. For the ANOVA, only one ear from each individual was selected at random.

RESULTS

The mean heights of the facial recess and extended facial recess at the stapes and round window levels are shown in Table 1. Forty-one left and 46 right ears were examined. There was no statistical difference between left and right ears at either the stapes or round window levels.

Effect of Sex. Combining children and adults, 51 temporal bones from males and 36 temporal bones

TABLE 3. SUMMARY OF ANALYSES OF VARIANCE IN 36 EARS IN 36 DIFFERENT ADULTS

Variable	*Sum of Squares*	*Degrees of Freedom*	*F-Value*	*p*
Outcome, mean facial recess at stapes				
Sex	0.013	1	0.04	.85
Pneumatization	0.001	1	0.00	.95
Interaction	1.104	1	3.23	.082
Error	10.944	32		
Outcome, mean facial recess at round window				
Sex	0.053	1	0.33	.57
Pneumatization	0.107	1	0.67	.42
Interaction	0.470	1	2.95	.095
Error	5.088	32		

from females were analyzed. The mean height of the facial recess at the stapes was 4.02 mm in males and 3.78 mm in females ($p<.05$ by univariate analysis). However, by ANOVA (Table 2) this sex difference was not statistically significant. The mean height of the extended facial recess at the round window was 3.01 mm in males and 2.93 mm in females, a difference that was not statistically significant.

Evaluating the possibility of a sex difference among adults only was performed by comparing 27 temporal bones of men and 20 temporal bones of women. The mean height of the extended facial recess at the stapes level was 4.07 mm for men and 3.83 mm for women, and at the round window level it was 3.11 mm for men and 2.88 mm for women. The differences in height at the stapes and round window were not significant (Table 3). The difference in mean height of the extended facial recess at the round window between males and females increases when the degree of pneumatization is controlled. Thus the mean height of the facial recess at the round window in adults with good pneumatization of the mastoid is significantly different ($p<.008$ by univariate analysis) for men (3.16 mm) and women (2.83 mm). However, the absolute differences are small compared to the ranges for men and women. This difference does not remain significant for adults with poor pneumatization.

Effect of Age. Forty-seven temporal bones from adults and 40 temporal bones from children were measured. The mean height of the extended facial recess at the stapes level was 3.97 mm and 3.86 mm respectively, and at the round window level, 3.01 mm and 2.93 mm respectively. At neither location was there a statistical difference between adults and children in regard to the heights of the extended facial recess.

Effect of Pneumatization. Of the adult temporal bones, 36 were judged to have good pneumatization and 11 were judged to have poor pneumatization. The height of the extended facial recess at the stapes level was 4.0 mm in those bones with good pneumatization and 3.87 mm in the bones with poor pneumatization. At the round window, the height was 3.0 mm in bones with good pneumatization and 3.05 mm in bones with poor pneumatization. Neither at the stapes nor at the round window level was there a statistically significant difference between the measurements in bones judged to have good and poor pneumatization.

DISCUSSION

Although there are differences in the height of the facial recess and extended facial recess with various groupings of variables (Table 1), the absolute differences are very small, especially compared to the range of individual variability. Furthermore, there were no significant differences in the height of the facial recess and extended facial recess at either the stapes or round window based on age of the patient or degree of pneumatization across all age groups. The measurements presented here at the stapes level are very similar to those found by Su et al,[4] who reported that the distance between the fallopian canal and the margin of the cartilaginous cushion of the tympanic annulus at the level of the pyramidal eminence was 4.01 mm, with a range of 2.4 to 5.7 mm. Similarly, these investigators did not find a significant change in size of the facial recess with an increase in age.

CLINICAL RELEVANCE

The results of this study demonstrate that the height of the extended facial recess, ie, the distance between the descending segment of the facial nerve and the annulus of the tympanic membrane, is very similar in all groups studied. The potential access, then, from the mastoid to the posterior tympanum should not be compromised significantly on the basis of sex, age, or degree of pneumatization. However, the degree of pneumatization, of both the central mastoid tract posteriorly and the facial sinus extension of the posterior tympanum anteriorly, may determine the relative ease of defining the potential spaces called the facial recess and the extended facial recess.

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TRANSMUCOSAL ELECTRICAL STIMULATION OF LARYNGEAL MUSCLES

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A new technique is described that enables discrete activation of individual laryngeal muscles by electrical stimulation across overlying mucosa. In 15 dogs, we defined six distinct motor points by transmucosal stimulation at 3 mA while observing the resulting characteristic position of the arytenoid and true vocal cord. Five dogs were then paralyzed with succinylcholine in order to simulate bilateral vocal cord paralysis. Application of a 3-mA stimulus at each motor point yielded no motion of the cords, but when the current was increased to 20 mA, characteristic responses were elicited. In five other dogs, botulinum toxin was injected directly into laryngeal muscles. Stimulation was used in an attempt to quantify the degree of neuromuscular blockade. In the last group of five dogs, we simulated cricoarytenoid arthritis by scarifying the joint. The extent and nature of the joint's impairment could be demonstrated by stimulation. Transmucosal stimulation appears promising as a clinical technique for correlating particular vocal cord movements and thresholds of activation with specific laryngeal disorders. Additionally, such a technique may be useful in clarifying how each laryngeal muscle acts upon the cricoarytenoid joint.

KEY WORDS — cricoarytenoid joint, laryngeal muscles, transmucosal electrical stimulation.

INTRODUCTION

Vocal cord movement disorders involve a wide spectrum of etiology and can be difficult to diagnose with precision. The laryngeal nerves and muscles may be intact, with motion restricted by entirely mechanical lesions. Some examples are vocal cord scarring, cricoarytenoid arthritis, and various infiltrative lesions. In contrast, the vocal cord may be mechanically normal, with motor deficits being the result of neuromuscular dysfunction involving upper or lower motor neurons, the neuromuscular synapse, or the muscle fibers themselves. Diagnosis sometimes can be made in the office setting from the patient's history and indirect laryngoscopy. Commonly, however, an accurate diagnosis requires a more thorough examination, which may involve palpation of the vocal cords under general anesthesia, computed tomography, stroboscopy, electromyography, and other specialized tests. Occasionally, the subtle nature of laryngeal movement disorders prevents diagnosis by even the most experienced clinician.

Indirect electrical stimulation of the main trunk of the recurrent laryngeal nerve (RLN) has been proposed as an aid in evaluating vocal cord mobility disorders. It has been demonstrated in dogs that the main trunk of the RLN can be stimulated by passing current across the tracheal wall,[1] esophageal wall,[1] or neck skin.[2,3] Activation of the RLN causes either abduction or adduction of the vocal cords, depending on the frequency of the applied stimulus. This technique may enable the clinician to test for continuity of the RLN and mechanical integrity of the larynx.

To add precision to this diagnostic tool, discrete excitation of individual laryngeal muscles was the focus of this study. Electric current was passed across the laryngeal mucosa in an effort to activate particular muscles. First, six motor points were identified. A motor point was defined as a distinct

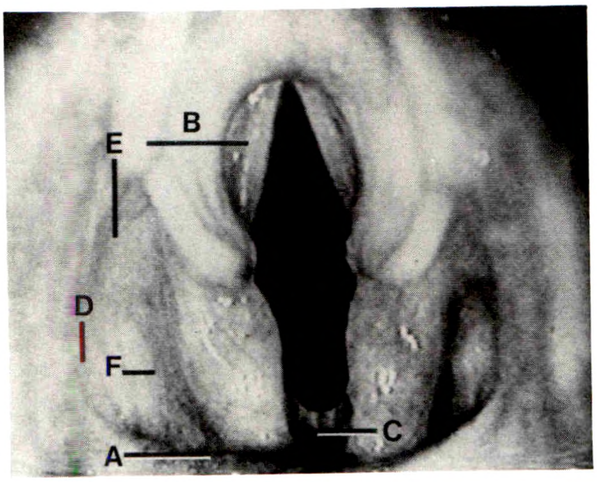

Fig 1. Motor points for transmucosal stimulation of laryngeal muscles. A — posterior cricoid area (corresponds to posterior surface of cricoid, which is not shown), B — membranous vocal cord area (includes undersurface of vocal cord), C — interarytenoid area, D — inferior pyriform area, E — superior pyriform area, F — medial pyriform area.

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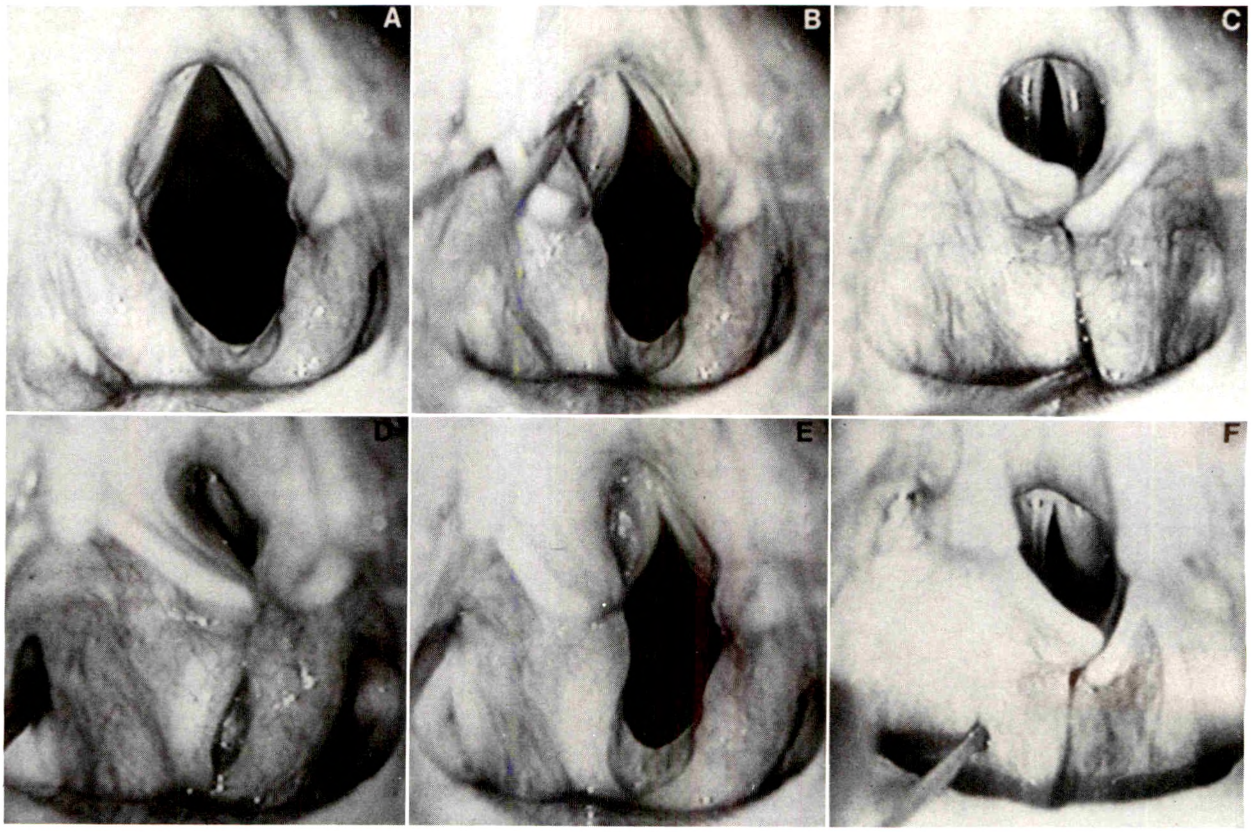

Fig 2. Transmucosal stimulation of normally innervated laryngeal muscles. Stimulation is delivered at 3 mA. Resting laryngeal configuration is shown in Fig 1. Note that each photograph corresponds to areas of stimulation demonstrated in Fig 1. A) Left posterior cricoarytenoid muscle stimulation. Probe enters postcricoid region at its lateral margin. B) Left direct thyroarytenoid muscle stimulation. Note bowing of left membranous vocal cord with no movement of arytenoid. To best demonstrate vocal cord bowing, photograph was taken during inspiration, when right vocal cord was abducting. C) Interarytenoid stimulation. Arytenoids are approximated closely throughout their medial margin, but membranous cords are relaxed and apart. D) Stimulation of nerve branch to left lateral cricoarytenoid muscle and thyroarytenoid muscle. Although majority of left vocal cord is forcefully adducted, posterior ends of arytenoids remain apart (compare to C and F). E) Stimulation of nerve branch to left thyroarytenoid muscle. Bowed appearance of muscle resembles that produced by direct stimulation in B. As in B, right vocal cord is abducted. F) Transverse arytenoid and ventricularis muscles. Posterior ends of arytenoids are approximated tightly, while membranous vocal cords are relaxed and apart.

area at which transmucosal stimulation consistently yielded a characteristic vocal cord response. Next, a variety of clinical situations were simulated experimentally. Under these experimental conditions, transmucosal stimulation was then applied in order to evaluate its effectiveness as a diagnostic aid.

MATERIALS AND METHODS

Fifteen male dogs, each weighing approximately 20 kg, were anesthetized with 30 mg/kg sodium pentobarbital and placed supine on the operating table. The mouth was stented open with a vaginal speculum and the epiglottis was pinned to the thyrohyoid membrane in order to maintain an unobstructed view of the vocal cords.

A monopolar stimulating probe was used, consisting of a blunted 1.25-mm stainless steel rod with insulation extending to within 1 mm of its tip. A second electrode was clamped to the buccal mucosa and acted as a distant anode. Current was generated by a biphasic DC stimulator (Recurrent Laryngeal Nerve Stimulator, WR Medical Electronics, Stillwater, Minn) which delivered pulses of 1 ms duration at 100 Hz. The dogs were continuously monitored by electrocardiography.

All dogs underwent laryngeal stimulation first in their innervated state. The probe tip was lightly positioned on the laryngeal mucosa while continuous pulsatile current was delivered. The probe was swept over all areas of accessible mucosa. Vocal cord motion was observed and recorded by video camera as amperage was increased from 1 to 5 mA. With experience, six discrete motor points were located that gave characteristic, reproducible vocal cord movement patterns (Fig 1).

Five dogs then underwent testing in the paralyzed state. Succinylcholine was injected, 1 mg/kg intravenously, to induce paralysis. Adequate neuromuscular blockade was defined as the complete absence of vocal cord motion when the main trunk of the RLN was stimulated supramaximally across the tracheal wall. Maintenance injections were administered every 15 minutes as needed. Ventilation was

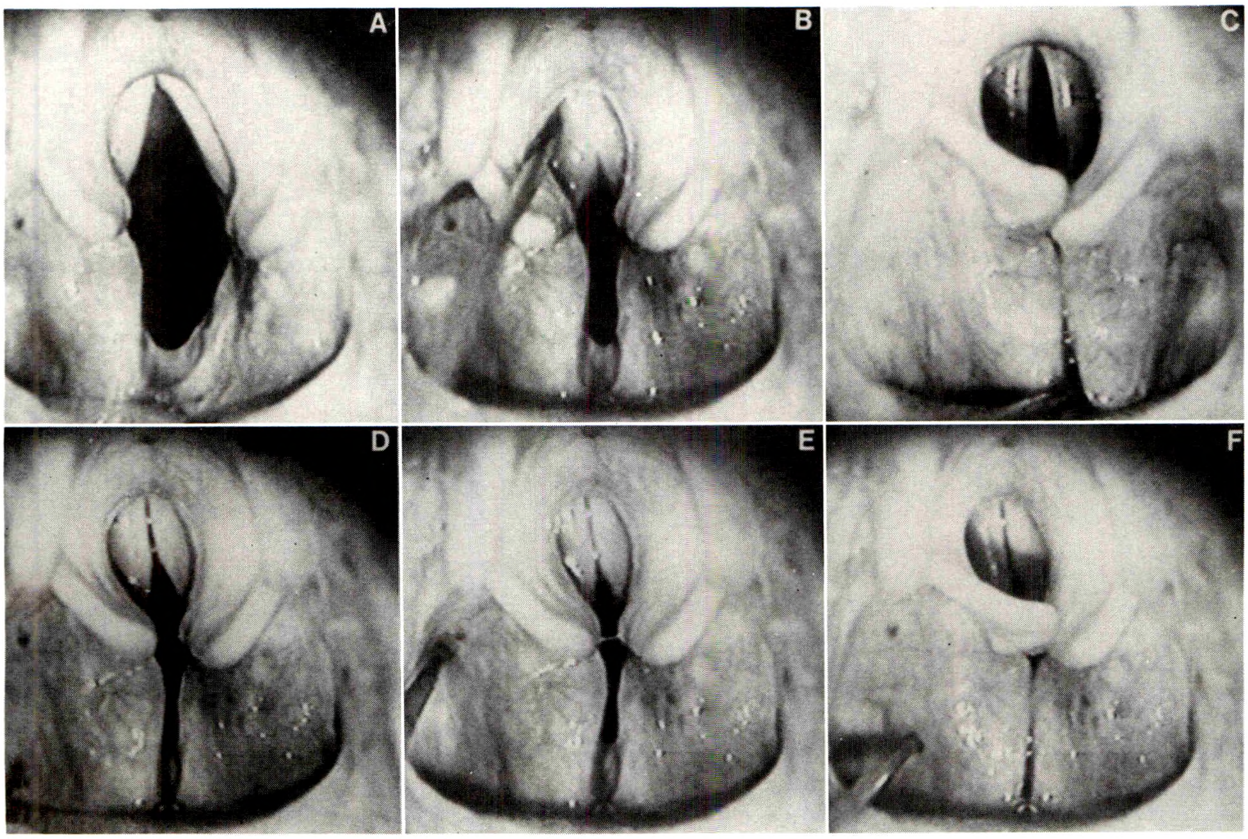

Fig 3. Transmucosal stimulation at 20 mA of paralyzed laryngeal muscles. Areas correspond to those of Figs 1 and 2. A) Right posterior cricoarytenoid muscle. B) Left thyroarytenoid muscle. Bowing resembles that in Fig 2B. Because animal was paralyzed, right vocal cord could not abduct. C) Interarytenoid stimulation. Position is identical to that of innervated larynx in Fig 2C. D) Inferior pyriform area. Moderate adduction of arytenoid suggests direct lateral cricoarytenoid muscle stimulation. Note dramatic difference in position when innervated larynx is stimulated at this area (Fig 2D). E) Superior pyriform sinus stimulation. Moderate adduction of arytenoid suggests direct lateral cricoarytenoid muscle stimulation. No bowing of membranous vocal cord occurs. Compare innervated larynx (Fig 2E). F) Medial pyriform sinus stimulation. Arytenoid adduction appears identical to that of innervated larynx (Fig 2F).

maintained via a cannulized tracheotomy. The stimulating probe was positioned on the previously defined motor points, and current was delivered at 100 Hz. Amperage was raised from 5 to 20 mA by 5-mA increments until vocal cord motion could be elicited.

In five other dogs, botulinum A toxin (Oculinum, Smith-Kettlewell Institute of Visual Sciences, San Francisco) was injected into the left transverse arytenoid and ventricularis muscles. Successive 15-U injections were made at 15-minute intervals. After each injection, the stimulating probe, carrying a 5-mA current, was swept over the muscle's mucosa. The effect of stimulation on vocal cord motion was observed. If any portion of the muscle remained active after 15 minutes, as demonstrated by either muscle tensing or cord motion, it was injected with another dose. After the entire muscle had been inactivated, the dog was paralyzed by intravenous injection of succinylcholine as described above. The complete neuromuscular paralysis thus achieved allowed a 20-mA current to be applied to the target muscle without inadvertent activation of surrounding structures. A 20-mA transmucosal stimulus was then applied to each motor point, and vocal cord response was noted. As a final test, the animals were reanesthetized the following day and retested at 5 mA.

For the last five dogs, cricoarytenoid joint fixation was produced using cauterization. A 16-gauge angiocatheter was passed through the cricothyroid notch and into the laryngeal lumen. The needle was advanced under direct vision until it made contact with mucosa overlying the cricoarytenoid joint. It was then pushed toward the joint until it contacted a cartilaginous surface. The needle was wired to an electrocautery so that the surface could be cauterized. Each dog's larynx was cauterized at a slightly different point, beginning in the interarytenoid area and moving anterolaterally. In three dogs, the needle was advanced through the joint capsule, and the inner joint surface was cauterized. After 1 month, the animals were reanesthetized and stimulated transmucosally at 1 to 5 mA.

RESULTS

Stimulation of Innervated Laryngeal Muscles. A characteristic vocal cord motion could be elicited by stimulating each of the six motor points (Fig 2).

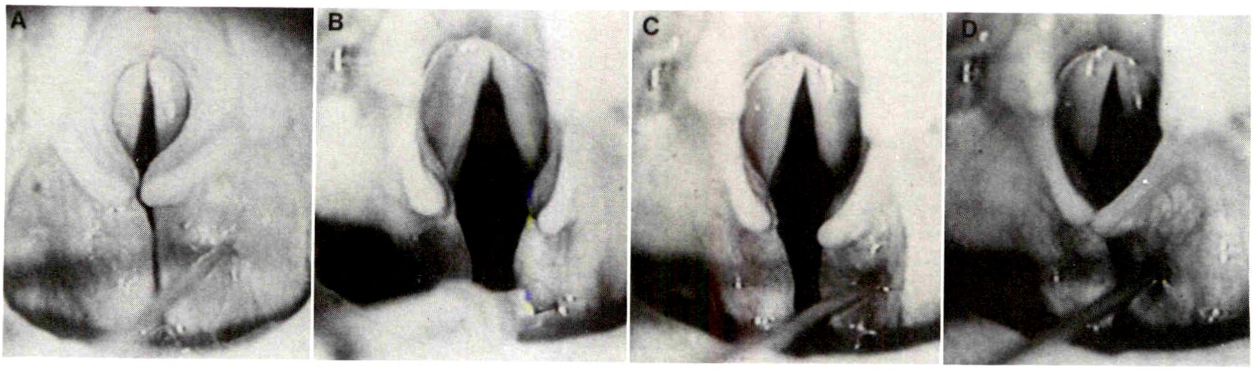

Fig 4. Transmucosal stimulation at 3 mA of medial pyriform sinus area before and after injection of botulinum toxin. A) Stimulation of medial pyriform area in normally innervated larynx. Movement is mirror image of that caused by stimulation of left side (Fig 1F). B) Botulinum toxin injection. Note that injection is occurring inferiorly. C) Stimulation near injection site. There is slight adduction, suggesting that some motor end plates near electrode are still intact. D) Stimulation away from injection site. By searching lateral surface of arytenoid, spot is found at which stimulation causes forceful adduction. This area will receive next injection.

Each specific pattern of movement was consistent for all dogs studied. Stimulation in the posterior cricoid region resulted in a wide abduction of the ipsilateral vocal cord (Fig 2A). Stimulating the superior surface of the membranous portion of the vocal cord caused a distinct bowing inward of the membranous cord (Fig 2B). Applying current to the interarytenoid area caused the arytenoids to closely approximate, while the membranous vocal cords remained relaxed and unapposed (Fig 2C). Stimulation of the inferior pyriform area resulted in ipsilateral vocal cord adduction, with the vocal process crossing the midline (Fig 2D). In the superior pyriform area, stimulation resulted in a bowing of the ipsilateral membranous vocal cord (Fig 2E). Finally, application of current to the medial surface of the pyriform sinus resulted in adduction of the involved arytenoid (Fig 2F).

Although some movement was evoked by stimulation at 1 mA, 3 mA always produced a discrete, identifiable motion. At current levels greater than 5 mA, neighboring muscles, particularly the cricothyroid and strap muscles, would contract. The resulting neck movements made observation difficult. At all amperages, stimulation elicited mucous discharge that periodically required removal. At no time were there any changes noted in mucosal integrity or color. Cardiac rhythm was also undisturbed by stimulation.

Use of Succinylcholine. Larynges paralyzed with succinylcholine demonstrated movement patterns similar to those of normal larynges, but required 20 mA before motion was evoked (Fig 3). At 20 mA, stimulation of the posterior cricoid area, membranous vocal cord, and interarytenoid and medial pyriform areas (Fig 3A,B,C,F) caused vocal cord motion identical to that seen in normal larynges. Stimulation in the deep pyriform area (Fig 3D,E) was much less dramatic than in the normal larynges. A 20-mA current produced no discernible erythema or edema of laryngeal mucosa.

Use of Botulinum. Injections of botulinum toxin into the lateral arytenoid area caused an immediate decrease in sensitivity to electrical stimulation (Fig 4). Areas adjacent to the injection site retained normal excitability at 5 mA, and subsequent injections were required. On average, three injections were required before inactivation was achieved. When a 20-mA current was applied, a motion pattern identical to that seen for the unparalyzed muscle occurred.

Retesting of the animal the following day invariably showed total unresponsiveness. However, when the animals were paralyzed with succinylcholine and stimulated at 20 mA, the entire lateral arytenoid area responded briskly.

Cricoarytenoid Scarring. The five dogs in which cautery had been applied to the cricoarytenoid joint area demonstrated drastically impaired vocal cord mobility (Fig 5). In four of the dogs, the joint appeared fixed, with minimal motion of the arytenoid regardless of where current was applied. Despite minimal arytenoid motion, the muscles could be seen to contract under the mucosa. This could be seen best at the membranous vocal cord area, where the distinctive bowing of the thyroarytenoid muscle could be evoked.

DISCUSSION

Interpreting the vocal cord motions elicited by transmucosal stimulation requires an understanding of electrical excitation of tissue and a knowledge of laryngeal anatomy and physiology.

When a normally innervated muscle is stimulated at low amperages, it is actually the neural elements within it that are activated by the current, rather than the muscle fibers directly. Muscle contraction occurs after transmission of the impulse across the synapse. Nerves have a lower activation threshold than do muscles. Therefore, in order to stimulate muscle fibers directly, either the muscle must be denervated, or neuromuscular transmission must be

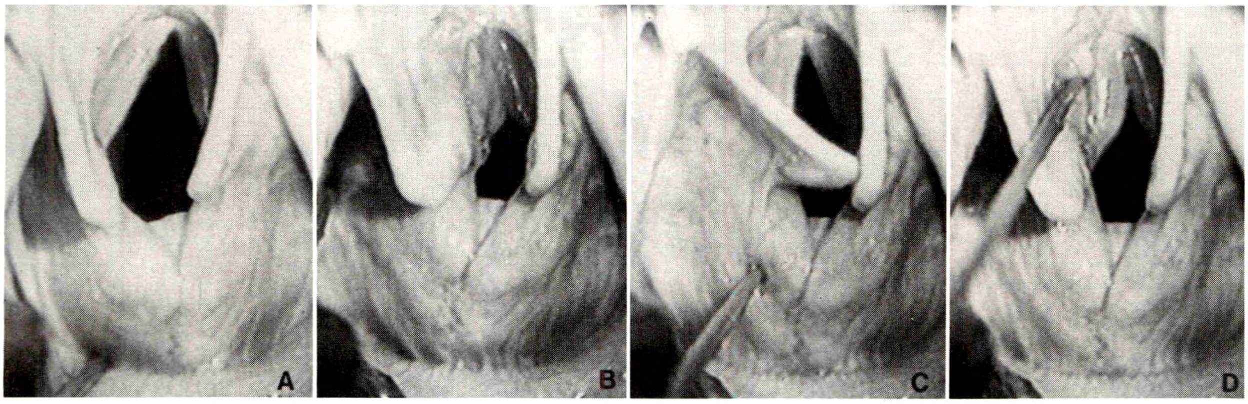

Fig 5. Transmucosal stimulation of larynx with posterior commissure scar. Stimulation is delivered at 3 mA. Note thick raised scar in posterior commissure and close apposition of posterior ends of arytenoids. A) Posterior cricoid area. Stimulation of left posterior cricoarytenoid muscle causes wide abduction of vocal process of left arytenoid. Note that scar prevents abduction of arytenoid body as is seen in normal larynx (Fig 2A). B) Inferior pyriform area. Stimulation of nerve elements to thyroarytenoid and lateral cricoarytenoid muscles would be expected to adduct forcefully left vocal cord (Fig 2D). In this case, scar prevents vocal cord adduction. Note effects of strong muscle contraction of left side: bowing of vocal cord, foreshortening of glottis, and rotation of posterior glottis. C) Lateral pyriform area. Stimulation of this area should adduct arytenoid bodies (Fig 2F). Scar prevents this from occurring. Note way in which ventricularis muscle adducts cuneiform cartilage and aryepiglottic fold across glottis. D) Membranous vocal cord. Bowing of membranous vocal cord shows that underlying thyroarytenoid muscle is capable of normal contraction.

blocked pharmacologically. Alternatively, the muscle can be stimulated at a location far from its midpoint, where the motor end plates are usually located.

In the larynx, where the muscles are relatively small, it is probably impossible to stimulate the muscle fibers directly without simultaneously activating the nerve fibers. Therefore, when the normal larynx is stimulated at 3 mA, it is reasonable to assume that only the neural elements in the region of the probe are being stimulated. From the underlying anatomy (Fig 6) it can be seen that in some areas (eg, area A) the target sites are individual muscles that are far enough away from each other and from other nerves that stimulation at 3 mA stimulates that individual muscle and the position of the vocal cord is directly the result of that single muscle contraction.

In area E, stimulation causes a lowering of the membranous vocal cord identical to that seen when the vocal cord is stimulated directly (Fig 2B,E). Presumably, the target in area E is entirely the nerve branch supplying the thyroarytenoid muscle. In area F, the transverse arytenoid muscle and the ventricularis muscle lie so close together that it is difficult to stimulate them separately. In area D, the lateral cricoarytenoid muscle lies beneath the terminal RLN branch to the thyroarytenoid muscle and therefore cannot be stimulated discretely. In area C, the target of stimulation is actually a cartilage, and the evoked movement depends on the current's spreading bilaterally to both transverse arytenoid muscles.

When the dogs were fully paralyzed with succinylcholine and stimulation was applied at 20 mA, the resulting vocal cord motion was assumed to be the result of direct muscle stimulation. Except in area D and E, the evoked movement is identical to that obtained by stimulating the innervated larynx at 3 mA. In area D, the forceful closure of the larynx is replaced by a modest adduction of the arytenoid body and vocal process, which presumably is the result of discrete lateral cricoarytenoid muscle activation. In area E, the bowing of the membranous cord seen in the normal larynx is replaced again by arytenoid adduction.

Local areas of paralysis due to botulinum toxin injection could be delineated easily by transmucosal stimulation. Finding areas of responsiveness in muscle helped direct the next injection until nearly total paralysis was achieved. An interesting finding regarding botulinum toxin was that over time, partial muscle paralysis often changed to total paralysis. The implication of this is that muscles that are merely to be weakened by botulinum toxin should be underinjected.

The last experimental situation involved cricoarytenoid muscle scarring. By use of stimulation, the extent to which the mobility of the arytenoid was impaired could be demonstrated easily. Even with total joint fixation, contractions of the underlying muscles could be seen as they strained against the immobile arytenoid.

Since the anatomy of the canine larynx is similar to that of the human larynx, transmucosal stimulation could be expected to function in clinical situations. For example, stimulation would be used during direct laryngoscopy on patients exhibiting vocal cord mobility disorders. Initially, stimulation would be applied when that patient was fully anesthetized but not paralyzed. Normal responsiveness and vocal cord movements would rule out lower motor neuron disease or joint immobility. Vocal cords that apparently moved well to palpation but were unresponsive to stimulation at low current would suggest denervation. Further evaluation

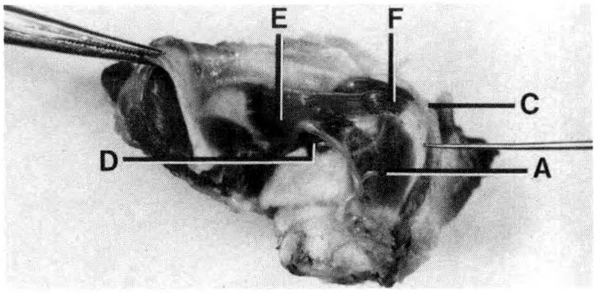

Fig 6. Laryngeal muscles and nerve branches that underlie stimulation target areas in excised canine larynx with left thyroid alae removed. Note course of recurrent laryngeal nerve along medial edge of posterior cricoarytenoid muscle (A), over lateral cricoarytenoid muscle (D), and then into thyroarytenoid muscle (E). In posterior cricoid area is posterior cricoarytenoid muscle (A). Note terminal nerve branch that enters muscle along its lateral margin. Also note nerve branch that travels underneath superior aspect of posterior cricoarytenoid muscle to supply muscles in area F. In membranous cord area is thyroarytenoid muscle (B). Superior surface of this muscle does not appear in this dissection. In interarytenoid area (C) dogs do not have interarytenoid muscle. Instead there is small cartilage into which parts of ventricularis muscle and transverse arytenoid muscle insert. As muscles from both sides insert on same freely mobile cartilage, movement of muscles of one side affects other side and therefore they function as continuous supraglottic sphincter. In inferior pyriform area is lateral cricoarytenoid muscle (D). Note that terminal branch of recurrent laryngeal nerve to thyroarytenoid muscle passes directly over lateral cricoarytenoid muscle. Also note that nerve branch to area F originates just proximal to this area. In superior pyriform area is lateral aspect of thyroarytenoid muscle (E). In medial pyriform area are ventricularis and transverse arytenoid (F) muscles. As noted above, nerve supply to this muscle travels underneath superior aspect of posterior cricoarytenoid.

would require total paralysis of the patient and stimulation at higher currents. A more specific use of this technique would be to guide the surgical therapy of the scarred larynx. During scar excisions, sequential testing of mobility by stimulation would allow tailoring of surgery so that maximum mobility would be achieved with minimal risk.

The ability of the larynx to be mobilized by stimulation may also find a role in airway control devices. Experiments have shown that stimulation delivered via indwelling prostheses allows controlled opening and closing of the glottis.[4] The abduction achieved by this method easily is capable of overcoming the strong adduction of laryngospasm.[5] It is possible that devices of this type may aid the anesthesiologist in intubating patients. Possibly some type of chronic prosthetic device might be useful in the prevention of aspiration in the neurologically impaired patient.

This experiment demonstrates that stimulation across mucosa can be used to evaluate accurately the function of underlying muscle. These techniques may find use in both diagnostic evaluation and therapeutic manipulation of other muscle groups along the upper respiratory tract. Examples include therapeutic stimulation of the tensor veli palatini muscle in patients with eustachian tube dysfunction, the cricopharyngeus muscle for swallowing disorders, and elevators of the palate and extensors of the tongue for sleep apnea.

An additional potential use of transmucosal stimulation is in reassessment of motion about the cricoarytenoid joint. The precise role of the various intrinsic muscles in manipulating the cricoid and arytenoid cartilages has long been an area of controversy. The classical anatomic studies of Negus[6] portray the arytenoid as a dual lever arm, with the intrinsic muscles acting on the muscular process to cause an axial rotation of the vocal process. The posterior cricoarytenoid muscle is regarded as the sole vocal cord abductor, pulling medialward on the muscular process and rotating the cord outward.[6]

Negus's theory has since been questioned by numerous investigators. A variety of anatomic and radiographic techniques have been used to reassess motion about this joint. Fink et al[7] combined an electromyographic study of the intrinsic laryngeal muscles with radiography of the larynx during the normal respiratory cycle. They proposed that abduction occurs not by axial rotation but by a posterior tilt of the arytenoids. This repositioning occurs through contraction of the sternothyroid muscle, which draws the larynx away from the hyoid and enlarges the anteroposterior diameter of the vestibule. This "bellows mechanism" hypothesis portrays the extrinsic muscles as the principal manipulators of the cords, with the intrinsic muscles mainly providing structural support.[7]

Frable,[8] using dissection of excised larynges and an elegant photographic apparatus, demonstrated the limitations of motion about the cricoarytenoid joint. Motion of the arytenoids was mapped in three planes. It was shown that the principal axis of rotation of the arytenoid on the cricoid is oblique, from dorsomediocranial to ventrolaterocaudal.[8]

Ardran and Kemp[9] extended the analysis of the anatomic constraints of movement about this joint using radiography of excised specimens. Calcific plaques within the cartilages were used as markers of movement while the glottis was manipulated by the experimenter. The arytenoids were found to rock forward and downward over a 40° arc on the long axis of the cricoid facets during passive adduction of the specimen.[9]

In a study that used anatomic dissection and electrical stimulation of freshly excised human larynges, Sellars and Keen[10] and Sellars[11] showed that the posterior cricoarytenoid muscle has three vectors of action that can move the arytenoid cartilage in three directions. The lateral vertical and oblique inferomedial fibers of the muscle rock the arytenoids backward, yielding abduction. The superomedial fibers pull the muscular process medialward and down, angling the vocal process upward and augmenting the abduction. A small amount of axial pivot also was noted.[10,11]

The transmucosal stimulation technique will enable an in vivo study of articular function. Radiographic visualization can be combined with discrete, noninvasive stimulation of each intrinsic muscle in order that each muscle's effect on cricoarytenoid joint positioning can be determined.

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THE SIXTH WORLD CONGRESS FOR BRONCHOLOGY

The Sixth World Congress for Bronchology will be held October 18-20, 1989, in Tokyo, and October 20-22, 1989, in Kyoto, Japan. For further information, contact Dr Masayuki Niitsuma, Secretary General, 6th World Congress for Bronchology, Department of Surgery, Tokyo Medical College, 6-7-1 Nishishinjuku, Shinjuku-ku, Tokyo 160, Japan; telephone 03-342-6111.

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AMERICAN AUDITORY SOCIETY

The 16th annual meeting of the American Auditory Society will be held in New Orleans on Sunday, September 24, 1989. The meeting is scheduled in conjunction with the annual meeting of the American Academy of Otolaryngology - Head & Neck Surgery. For further information, contact J. W. Hall III, PhD, Department of Otolaryngology, School of Medicine, Vanderbilt University, Nashville, TN 37232.

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TEMPORAL BONE COMPUTED TOMOGRAPHY IN THE PREOPERATIVE EVALUATION FOR COCHLEAR IMPLANTATION

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High resolution computed tomography of the temporal bone is performed routinely in the preoperative evaluation for cochlear implantation. A retrospective review was performed to determine the effect of these studies. The clinical significance was determined through consultation with the referring otolaryngologist and review of the operative reports. Twelve examinations were reviewed. Abnormalities were identified in 12 of the 24 ears examined (eight of 12 patients studied). In two patients the CT examination strongly influenced the selection of the ear to be implanted. In four additional cases information felt to be useful in preoperative planning was provided. Useful information related to potential complications with the mechanical insertion of the electrode apparatus. We conclude that temporal bone abnormalities are common in the population undergoing cochlear implantation and that high resolution temporal bone CT is essential in preoperative evaluation. The CT scan should be interpreted with adequate knowledge of the operative procedure to evaluate any possible barriers to the mechanical insertion of the internal components of the system.

KEY WORDS — computed tomography of temporal bone, radiography of temporal bone.

INTRODUCTION

The multichannel cochlear implant has been shown to be useful in restoring hearing to the profoundly deaf individual unable to derive significant improvement from the use of conventional hearing aids. Identification of environmental sounds and improvement in communication both with and without the use of lipreading has been documented following insertion of this device.[1,2] The components of this system include 1) a speech processor that converts environmental sound into an electrode coding strategy, 2) a headset that contains an ear level microphone and a device for transmission of the signal from the speech processor to the receiver/stimulator, 3) a receiver/stimulator that receives the signal from the headset and transmits it to the intracochlear electrode array, and 4) the intracochlear electrode array itself, which stimulates the cochlear nerve.[1-4] The speech processor and headset form the external portion of this system and are connected postoperatively. The receiver/stimulator and the intracochlear electrode array are implanted surgically (Fig 1). The basis for this study is the role of computed tomography in the preoperative evaluation for implantation of the internal components of the system.

MATERIALS AND METHODS

The patients were referred to the University of Iowa for evaluation of profound bilateral deafness. In all cases the onset of deafness was after speech development. The average hearing threshold at 500, 1,000, and 2,000 Hz was equal to or worse than 95 dB. No significant improvement was identified with the use of external hearing aids.

Preoperatively each patient received a high resolution CT examination of the temporal bones. The Siemens Somaton DRH was used for nine cases, and the Picker 1200 for three cases. Images were obtained parallel to the canthomeatal line. Slice thickness was 1.0 mm and the slice interval was 1.0 mm. The images and operative reports were reviewed

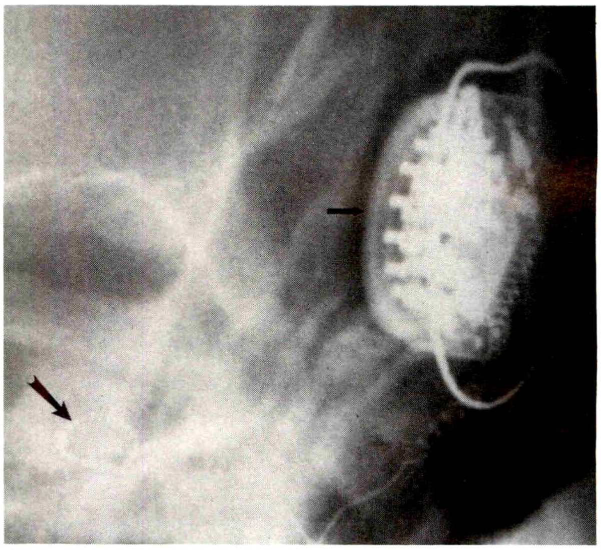

Fig 1. Plain film examination of internal components of cochlear implant. Receiver/stimulator resides in bed created in parietotemporal region (small arrow). Intracochlear electrode array is located in basal turn of cochlea (large arrow).

From the Departments of Radiology (Mueller, Dolan) and Otolaryngology-Head and Neck Surgery (Gantz), University of Iowa Hospitals and Clinics, Iowa City. Supported by research grant NS 20466-03 from the National Institutes of Health/National Institute of Neurological and Communicative Disorders and Stroke and grant RR 59 from the General Clinical Research Centers Program, Division of Research Resources, National Institutes of Health.

REPRINTS — Kenneth D. Dolan, MD, Dept of Radiology, University of Iowa Hospitals and Clinics, Iowa City, IA 52242.

TABLE 1. ABNORMALITIES IDENTIFIED BY COMPUTED TOMOGRAPHY IN 12 OF 24 TEMPORAL BONES EXAMINED

Abnormalities	*No. of Temporal Bones*
Otosclerosis	4
Prior mastoidectomy	2
Mondini deformity	2
Cochlear ossification	2
Soft tissue in round window niche	1
Dislocated incus	1
Total	12

Total number of patients was 12.

retrospectively with the referring otolaryngologist. Tabulation was made of 1) the total number of abnormalities identified, 2) the abnormalities strongly affecting the choice of ear to be implanted, and 3) examinations in which the information did not lead directly to the choice of the temporal bone for implantation, but in which it was felt to be useful in preoperative planning.

RESULTS

Abnormalities were identified in 12 of the 24 ears examined. This represented abnormalities in eight of the 12 patients studied. Abnormalities included cochlear otosclerosis, the Mondini deformity, cochlear ossification, and evidence of prior mastoidectomy (Table 1). In two cases identification of these abnormalities strongly influenced the selection of the temporal bone for implantation. In an additional four cases the CT examination was felt to provide useful preoperative information (Table 2).

TABLE 2. EFFECT OF PREOPERATIVE COMPUTED TOMOGRAPHY IN 12 PATIENTS

Effect		*No. of Patients*
Strongly influenced selection of ear for implantation		
Sclerosis of basal turn	1	
Prior mastoidectomy (radical mastoidectomy anatomy)	1	
Total		2
Provided useful preoperative information		
Cochlear otosclerosis with narrowed basal turn	1	
Cochlear otosclerosis	1	
Mondini deformity	1	
Prior mastoidectomy (small cochlear lumen, soft tissue in middle ear cavity, simple mastoidectomy anatomy)	1	
Total		4

DISCUSSION

Temporal bone abnormalities have been reported as being common in the deaf population.[5] Our find-

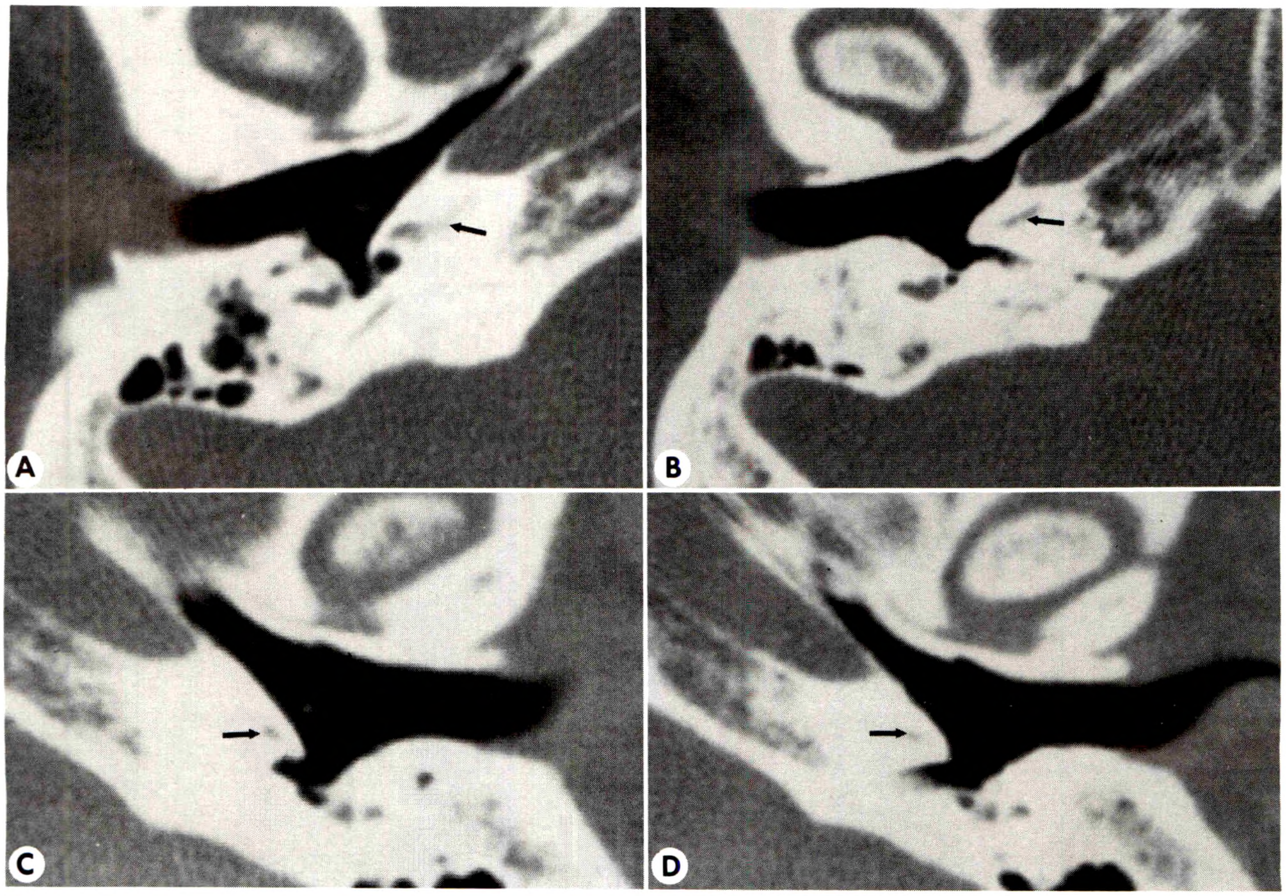

Fig 2. Bilateral computed tomographic examinations of basal turn of cochlea (arrows). Significantly less ossification is identified on A,B) right than on C,D) left.

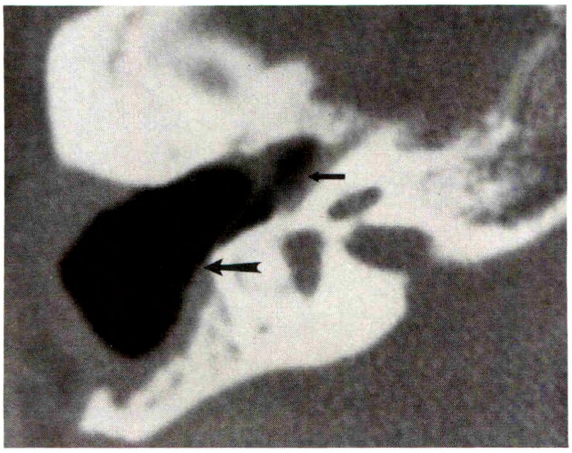

Fig 3. Absence of posterior wall of external auditory canal (large arrow) and ossicles in middle ear cavity (small arrow). These findings are consistent with prior radical mastoidectomy.

ings agree with this conclusion. Abnormalities were identified in eight of the 12 patients studied and in 12 of the 24 temporal bones examined. The significance of these abnormalities with respect to cochlear implantation depended on their possible interference with normal insertion of the internal components of the implantation device. To assess adequately the possibility of complications to the insertion process, the radiologist's knowledge of the surgical procedure is mandatory.

The receiver/stimulator component is placed within a bed created in the mastoid, parietal, and/or occipital bones. It has been recommended that the thickness of the parietal bone be measured preoperatively as an aid to the implantation of this device.[6] Implantation of the intracochlear electrode array occurs through the following steps. A complete mastoidectomy is performed. Then the middle ear cavity is entered through a facial recess approach. Identification of the round window niche is made. The scala tympani then can be entered either by direct access via the round window or through a cochlear fenestration. Cochlear fenestration is performed in a location anterior and inferior to the round window niche. Therefore, mechanical barriers to electrode insertion should be searched for in the mastoid air cells, facial recess, middle ear cavity, and promontory. Identification of the round window niche and patency of the cochlear canal should be documented.

In our population, two cases were identified in which abnormalities were of sufficient magnitude to play a significant role in the determination of the ear to be operated upon. In the first case, asymmetry in degree of sclerosis of the basal turn of the cochlea was present. It was felt that the ear demonstrating the lesser degree of sclerosis offered a higher probability for successful implantation, and this ear was chosen (Fig 2). In the second case, a mastoidectomy had been performed previously. The CT examination demonstrated anatomic changes consistent with a radical mastoidectomy (Fig 3). In the absence of the posterior wall of the external auditory canal, adequate protection and support of the electrode was not available. The contralateral temporal bone therefore was selected.

In four additional cases information useful in preoperative planning was identified. Cochlear otosclerosis was identified in two of these cases. This information aided the surgeon by calling attention

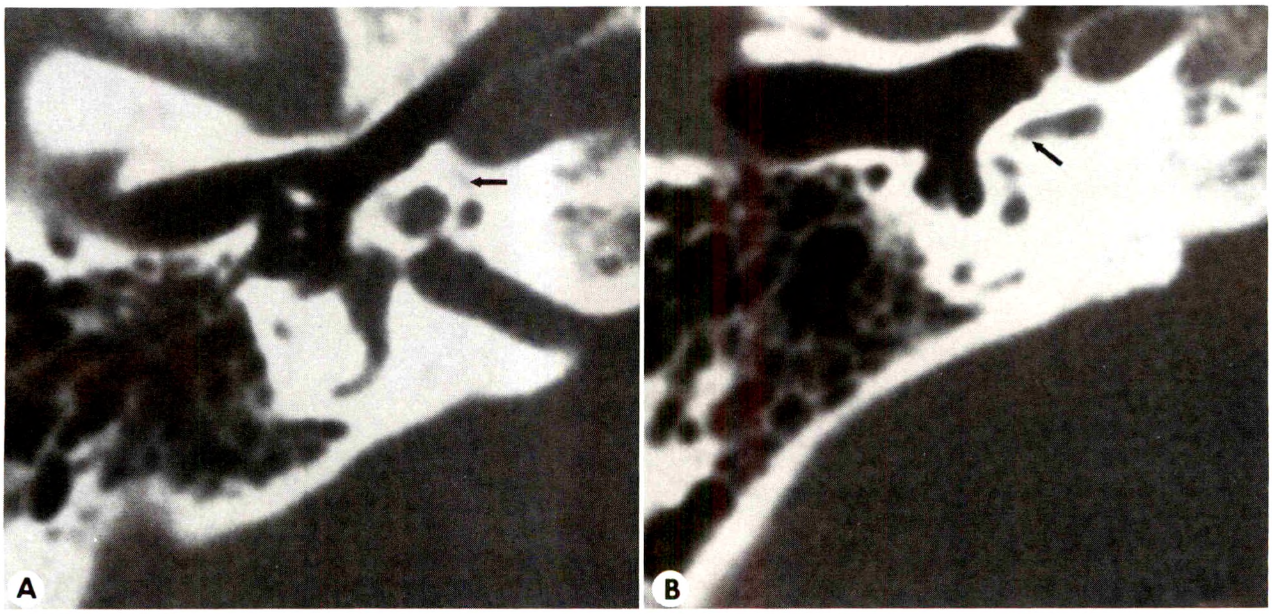

Fig 4. A) Focus of decreased attenuation in otic capsule consistent with otosclerosis (arrow). B) Narrowed segment of proximal basal turn of cochlea (arrow).

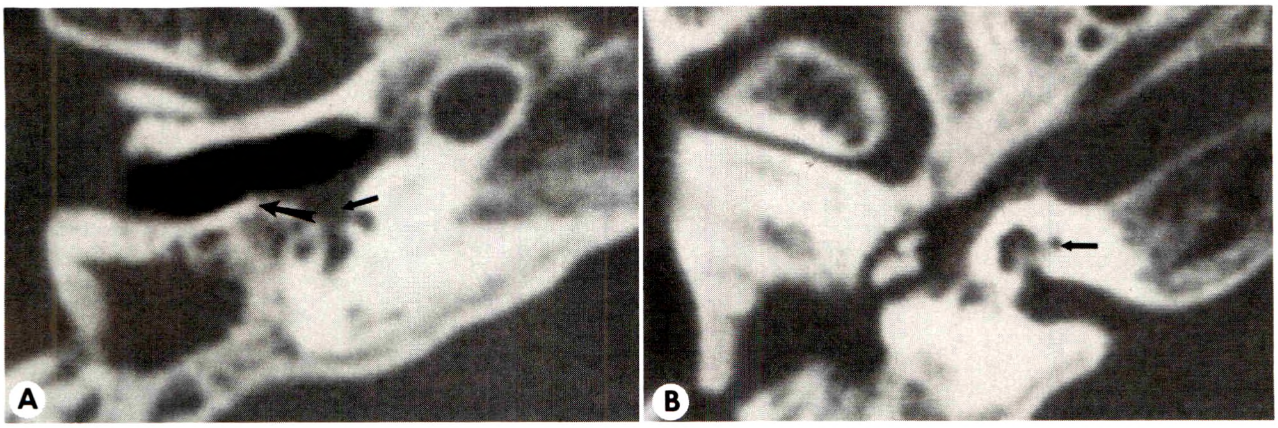

Fig 5. Temporal bone showing result of simple mastoidectomy. A) Intact posterior wall of external auditory canal (large arrow) and soft tissue covering round window niche (small arrow) are identified. B) Small lumen of cochlea is noted (arrow).

to the possibility of increased depth of drilling being needed to reach the scala tympani. In addition, in one of these cases evidence of a narrowed segment of the proximal basal turn was identified. This aided the surgeon in the choice of cochlear fenestration sites (Fig 4).

Mondini deformities were identified bilaterally in our third case. Profuse perilymph flow has been reported at the time of cochlear fenestration in a patient with this abnormality.[7] Preoperative knowledge of this deformity allowed the surgeon to prepare adequately for this possibility. The fourth case was that of a patient with a prior history of meningitis and a previous mastoidectomy. Selection of the ear for implantation was made on the basis of infection in the opposite ear. Preoperative examination demonstrated anatomic changes consistent with a simple mastoidectomy. Thus, the posterior wall of the external auditory canal was intact. Soft tissue density was identified covering the round window niche. The usual surgical landmarks therefore could be obscured. Finally, the diameter of the cochlea appeared small (Fig 5). This proved to be of extreme importance. The surgeon was initially unable to pass the electrode into the scala tympani. This necessitated drilling out the first and second cochlear turns.

In summary, radiographically identifiable abnormalities are common in the patient population undergoing cochlear implantation. A knowledge of the implantation technique is felt to be necessary to assess adequately the significance of the abnormalities identified to the implantation procedure. High resolution technique (algorithm) CT with thin sections contiguous to one another and a bone window are imperative to producing appropriate views for preoperative study. Only this form of CT examination will show the important anatomic or pathologic surgical details we have described in this report.

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INCREASED CILIARY BEATING FREQUENCY OF NASAL MUCOSA FOLLOWING IMMUNOTHERAPY FOR ALLERGY

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The present study was designed to elucidate the effect of immunotherapy on the beating frequency of nasal cilia in patients with nasal allergy. Of 40 patients with nasal allergy due to *Dermatophagoides farinae*, 20 were treated with immunotherapy by the use of *D farinae* extracts, and 20 control patients were treated with antihistamine tablets. Mucosal pieces were taken from the right inferior turbinate before and 1 year after the initiation of treatment, and the ciliary beating frequency (CBF) was examined by a photoelectric method. The use of antihistamine tablets did not increase CBF even when it relieved the nasal symptoms. Immunotherapy, on the other hand, increased CBF in 66.7% of patients when it alleviated their nasal symptoms. The CBF before immunotherapy of patients who showed an increased CBF after treatment was statistically higher than that of patients who did not.

KEY WORDS — ciliary beating frequency, immunotherapy, nasal allergy.

INTRODUCTION

Nasal allergy is a disease ensuing from type 1 allergic reaction, in which the most significant pathogenetic factor is an interaction between allergens and their relevant IgE antibodies.[1-3] Recent studies suggest that allergen-specific IgG4 antibodies act as blocking antibodies because they produce favorable effects following hyposensitization, or immunotherapy in current terminology.[4-6] We previously determined the level of IgG4 antibodies in sera of patients with *Dermatophagoides farinae*–sensitive nasal allergy before and after immunotherapy.[7] We found in the study that induction of IgG4 antibodies relieved nasal symptoms in most cases. In some cases, however, an increased level of IgG4 antibodies failed to relieve nasal symptoms. Not all of the clinical problems involved in nasal allergy seem to be solved through an immunologic means alone. Nonspecific disorders of nasal cavity mucosa following immunologic reactions might be implicated in the clinical manifestation of the disease.

Immunotherapy is one of the most effective treatments for patients with nasal allergies.[8] It is well established that immunotherapy can reduce nasal allergen sensitivity. However, the effect of immunotherapy on the nonspecific disorders of nasal allergy mucosa has received less consideration. The present study was designed to elucidate the effect of immunotherapy on the beating frequency of nasal cilia in patients with nasal allergy.

MATERIALS AND METHODS

This study was performed in 40 patients who satisfied completely the following criteria: 1) typical nasal allergy symptoms of the perennial type; 2) no seasonal nasal allergy such as hay fever; 3) positive reactions involving immediate hypersensitivity skin test with house dust mite (*D farinae*), nasal provocation test with *D farinae*, or nasal eosinophilia; 4) no marked septal deviation or nasal polyps; and 5) absence of any previous treatment for allergy. The testing and the severity classification were performed according to Okuda et al[9,10] with some modifications. In brief, subjective symptoms were assessed by a questionnaire in which sneezing, nose blowing, and blockage were graded on a four-point scale: none, mild, moderate, and severe (Tables 1 and 2). Eosinophils in nasal secretions obtained from nose blowings were stained by Hansel staining and semiquantitated. For nasal provocation tests, a paper disc containing *D farinae* was placed on the inferior turbinate and changes (sneezing and itching, mucosal swelling, and watery secretions) occurring in 10 minutes were recorded (Table 3).

Two weeks after the allergy testing was completed in each patient, six pieces of mucosal epithelium were obtained from each patient by gentle scraping with a blue curette on the central part of the right inferior turbinate. Mucosal pieces were kept in a medium containing RPMI 1640 solution[11-13] prior to examination. The ciliary beating frequency (CBF) per minute in the most active cell of each mucosal piece was determined in a physiologic saline solution at an ambient temperature of 30°C, by use of the direct and quantitative photoelectric method of Ohashi and Nakai.[14,15] Each mucosal piece was measured five times, and the average value was used.

The 40 patients, who entered this trial with prior informed consent, were divided into two groups according to their own choice. Hyposensitization, or

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TABLE 1. EVALUATION OF SEVERITY OF EACH OF THREE NASAL SYMPTOMS

	Severe	*Moderate*	*Mild*	*None*
Sneezing (average number of attacks per day)	≥ 11 times	6-10 times	< 6 times	0 times
Nasal discharge (average number of nose blowings a day)	≥ 11 times	6-10 times	< 6 times	0 times
Nasal blockage	Predominant mouth breathing	Occasional mouth breathing	No mouth breathing, but sensation of blockage	No mouth breathing or sensation of blockage

immunotherapy in current terminology, was performed on 20 patients (immunotherapy group). They received weekly injections of *D farinae* extract (Torii, Tokyo). Injections were started one dilution below the weakest giving a positive test. If no systemic or large local reaction was noted, the next injection was increased by 50% until 0.3 mL of 1:1,000 *D farinae* extract was tolerated. This was estimated to take 20 to 30 weeks to accomplish. After the highest dosage was reached, the patients were maintained at this dosage weekly, and then administration was adjusted slowly to 1-, 2-, 3-, and finally 4-week intervals. All adverse reactions to injection were monitored carefully. Patients who received immunotherapy were instructed to avoid additional treatments for their allergy. The remaining 20 patients, who rejected immunotherapy (control group), were treated by antihistamine tablets.

About 1 year (between 12 and 13 months) after the initiation of immunotherapy or antihistamine treatment, six pieces of mucosal epithelium were obtained from the same location in each of the 40 patients in the same manner. The CBF per minute of each epithelial piece was determined by the same photoelectric method. The data obtained concerning CBF were analyzed statistically by Student's *t* test.

The clinical improvement brought about by immunotherapy or antihistamine tablets was evaluated according to the criteria of Okuda et al.[9,10] Clinical improvement was divided into two types: good response (moderate improvement or better according to the criteria of Okuda et al) and poor response.

RESULTS

BACKGROUND OF PATIENTS BEFORE TREATMENT

Age. The control group consisted of eight children (6 to 14 years old) and 12 adults (over 20 years old). The immunotherapy group also consisted of eight children and 12 adults. There was no difference between the two groups in the ages of patients.

Severity. Each group represented two mild, 14 moderate, and four severe allergies. No difference in the severity of nasal allergy was recognized between the two groups.

Ciliary Beating Frequency. The respective CBFs per minute before the treatment were 530 ± 74 (mean ± SD) in the control group and 522 ± 112 in the immunotherapy group. There was no significant difference in CBF between the two groups.

CLINICAL IMPROVEMENT

In the control group, nine patients (45%; five of eight children and four of 12 adults) showed a good response to antihistamine tablets, and the remaining 11 patients (55%) showed a poor response. To immunotherapy, 15 patients (75%; eight of eight children and seven of 12 adults) showed a good response, and the remaining five patients (25%) showed a poor response. Hence, a greater clinical improvement was observed in the immunotherapy group than in the control group.

CHANGE IN CILIARY BEATING FREQUENCY

No patients of the control group showed significant changes in CBF due to the treatment (Table 4). The use of antihistamine tablets had no effect on CBF even when it achieved clinical benefit for the nasal symptoms. On the other hand, ten patients (Nos. 3, 5, 7, 8, 11, 14, 15, 16, 18, 20) of the immunotherapy group showed a significantly increased CBF after the treatment. However, immunotherapy did not change the level of CBF in the remaining ten patients. Although all the children of the immunotherapy group showed an increased CBF after the treatment, only two of the 12 adults (16.7%) demonstrated an increased CBF after the same treatment. No patient who demonstrated a poor response to the immunotherapy showed an increased CBF. On the other hand, ten of the 15 patients (66.7%) who demonstrated a good response to the immunotherapy showed an increased CBF after the treatment. In addition, the mean CBF before immunotherapy of patients who obtained an increased CBF was 604 ± 57, and that of patients who did not

TABLE 2. OVERALL SEVERITY OF NASAL SYMPTOMS

	*Sneezing and/or Nose Blowing**		
*Blockage**	*< 6/d*	*6/d to 10/d*	*≥ 11/d*
Mild	1	2	3
Moderate	2	2	3
Severe	3	3	3

1 — mild, 2 — moderate, 3 — severe.

*See Table 1 for severity scales.

TABLE 3. GRADE OF ALLERGY TEST RESULTS

Intradermal Test	*A (41-mm Erythema, 16-mm Wheal)*	*B (21- to 40-mm Erythema, 10- to 15-mm Wheal)*	*C (21- to 40-mm Erythema, 9-mm Wheal)*	*D (20-mm Erythema, 9-mm Wheal)*
Nasal provocation test*	3 symptoms, ≥6 sneezes	3 symptoms, <6 sneezes	2 symptoms	1 or no symptoms
Nasal eosinophilia	Clustered	Mild to moderate	Scattered	None

*Three symptoms are sneezing and itching, mucosal swelling, and watery secretion.

obtain an increased CBF was 440±91. Statistical analysis showed a significant difference between the two levels ($p < .10$).

DISCUSSION

Mucociliary function is an important primary defense mechanism of the respiratory tract. It protects the host by trapping and sweeping away inhaled particles, including allergens. The speed of mucus transport in healthy humans lies within a range of 4 to 10 mm/min in the trachea[16] and 0 to 14.8 mm/min in the nose.[17] Patients with bronchial asthma have an impaired mucociliary transport.[18,19] In young asymptomatic nonsmoking subjects with allergic bronchial asthma the mean baseline tracheal mucus velocity is 54% of that in normal nonsmokers, and inhalation challenge with specific antigen further reduces tracheal mucus velocity by

TABLE 4. CLINICAL STATUS AND CILIARY BEATING FREQUENCY BEFORE AND AFTER TREATMENT

Patient No.	*Age*	*Severity of Nasal Symptoms*	*Clinical Improvement*	*CBF (Mean ± SD) Before*	*CBF (Mean ± SD) After*	*Significance of Change in CBF*
Immunotherapy group						
1	A	Moderate	Good	393±36	390±39	NS
2	A	Moderate	Good	415±33	470±36	NS
3	C	Mild	Good	660±29	737±33	p<.10
4	A	Moderate	Poor	462±37	435±36	NS
5	A	Moderate	Good	602±37	672±20	p<.10
6	A	Severe	Poor	387±30	375±25	NS
7	C	Moderate	Good	647±43	732±33	p<.10
8	C	Moderate	Good	602±29	710±32	p<.05
9	A	Moderate	Good	463±46	452±43	NS
10	A	Severe	Poor	370±30	393±29	NS
11	C	Moderate	Good	607±33	727±31	p<.025
12	A	Moderate	Good	543±30	568±26	NS
13	A	Moderate	Good	390±34	415±39	NS
14	C	Mild	Good	687±25	775±27	p<.05
15	C	Moderate	Good	540±18	635±37	p<.05
16	C	Severe	Good	493±38	628±34	p<.025
17	A	Moderate	Poor	641±32	640±28	NS
18	A	Moderate	Good	623±35	722±31	p<.05
19	A	Severe	Poor	340±20	347±34	NS
20	C	Moderate	Good	583±26	693±31	p<.05
Control group						
21	A	Severe	Poor	452±33	440±23	NS
22	C	Moderate	Good	607±27	611±31	NS
23	C	Moderate	Poor	483±35	445±35	NS
24	C	Moderate	Poor	544±31	490±37	NS
25	C	Moderate	Poor	492±36	452±37	NS
26	A	Severe	Poor	387±41	396±38	NS
27	A	Moderate	Good	596±31	572±36	NS
28	C	Moderate	Good	642±38	653±35	NS
29	A	Moderate	Good	458±33	468±36	NS
30	A	Severe	Poor	592±31	622±41	NS
31	C	Moderate	Good	396±38	376±33	NS
32	A	Mild	Good	572±41	505±31	NS
33	A	Moderate	Good	622±38	603±31	NS
34	C	Mild	Good	583±27	555±36	NS
35	C	Moderate	Good	581±33	584±35	NS
36	A	Moderate	Poor	495±40	475±35	NS
37	A	Moderate	Poor	454±38	469±35	NS
38	A	Severe	Poor	512±33	533±47	NS
39	A	Moderate	Poor	603±40	607±37	NS
40	A	Moderate	Poor	544±29	540±32	NS

CBF — ciliary beating frequency, A — adult (>20 years), C — child (6 to 14 years), NS — not significant.

28%.[20] Since nasal allergy is a type of allergic disease in the respiratory system like asthma, patients with nasal allergy might have an impaired mucociliary velocity in the nose. Direct evidence suggesting an impaired mucociliary velocity in the patient with nasal allergy has never been established; however, indirect evidence is accumulating. In human subjects with chronic nasal allergy, the number of ciliated cells is diminished, and the structure of ciliated cells is affected.[21,22] The morphologic basis of the nasal mucociliary system is the ciliated cell and the mucus covering it, and the ciliary beating is an integral component of mucociliary transport. Pathologic ciliated cells display a reduced CBF,[23-25] and the nasal CBF of patients with nasal allergy is reduced as the morbid period is prolonged.[26] It is conceivable that recurrent allergic reactions might affect ciliated cells and CBF. Although many factors are involved in mucociliary transport, including the mucus viscosity, the thickness of the mucus blanket, and the depth of the periciliary layer, as well as CBF, reduced CBF could result in an impaired mucociliary transport velocity and prolonged contact of allergens and IgE antibodies.

Immunotherapy is one of the most effective treatments for patients with nasal allergy because of the production of specific IgG4 antibodies.[4-7] The possibility cannot be ruled out that immunotherapy relieves the mucociliary disorder of the nasal allergy mucosa. To the best of our knowledge, however, the effect of immunotherapy on the nasal mucociliary disorder has never been investigated. In our present study, CBF before and after immunotherapy was examined by our photoelectric method.[14,15] To serve as a control, CBF before and after an antiallergic treatment using antihistamine tablets also was examined. The use of antihistamine tablets did not increase CBF, even when it relieved nasal symptoms. On the other hand, the immunotherapy increased CBF in 66.7% of patients when it relieved their nasal symptoms; no patients who demonstrated a poor response to the immunotherapy had an increased CBF. An inference could be drawn from the data that immunotherapy increases nasal CBF only when it alleviates nasal symptoms. Therefore, it may be assumed that an increased CBF is due to a reduction in nasal allergen sensitivity following immunotherapy. All the children treated with immunotherapy demonstrated an increased nasal CBF, but only 16.7% of adults had an increased CBF after immunotherapy. In addition, the CBF before immunotherapy of patients who showed an increased CBF was statistically higher than that of patients who did not. These findings may be interpreted as follows. There is a close relationship between the fine structure of ciliated cells and CBF,[23] and recovery of an impaired CBF is possible on the condition that the ciliated cell is not involved in a morphologically irreversible lesion.[27-30] On the basis of these studies,[23,27-30] it appears that the disorder of the nasal mucosa of the patients who failed to increase their CBF following immunotherapy was already irreversible.

In conclusion, it has been shown by the present study that immunotherapy for allergy can increase the CBF of nasal mucosa, especially in children, when it alleviates their nasal symptoms. The CBF is an index of the mucociliary transport velocity, and an increased CBF might result in an accelerated mucociliary transport velocity. However, other factors, including physiologic properties of the mucus blanket, and coordination of the mucus blanket and CBF, should be taken into consideration for more explicit understanding. In this respect, further study will be needed before we can explain fully the effect of immunotherapy on the mucociliary activity of nasal mucosa.

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GASEOUS PATHWAYS IN ATELECTATIC EARS

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KFAR SABA, ISRAEL

Thirteen atelectatic ears were politzerized with CO_2, O_2, air, and N_2. In consequence, the atelectasis in these ears disappeared, only to reappear again slowly. The reappearance of retraction corresponded in speed to the diffusion coefficient of the gases, indicating a regular diffusion process. At the same time, four of these politzerized patients were able to collapse the tympanic membrane of their air-filled middle ears abruptly through sniffing or swallowing. At least three patients could introduce air actively and voluntarily into their ears. These observations indicate that in atelectatic ears, as in normal ears, air can enter and leave the middle ear through one of two routes. One is the eustachian tube, through which air can pass both ways as a bolus. The second route is through the bloodstream, which gases enter and leave according to the rules of biologic diffusion. While the origin of negative pressure in atelectatic ears is unknown, this study shows that it may be a complex process stemming from a quantitative imbalance of loss versus gain of gas entry through either of the two routes.

KEY WORDS — aeration deficiency, diffusion of gases, eustachian tube.

The classical concept concerning middle ear aeration has it that under physiologic conditions oxygen is absorbed continuously from the middle ear (ME) into the bloodstream. The gas deficit created thereby is compensated continuously by reentry of air through the eustachian tube (ET).[1] Air is considered to pass through the ET when swallowing takes place. Several pathologic conditions, of which atelectasis, retraction pockets, and secretory otitis media are the main representatives, are presumed to cause a defect in the return of air into the ME, leading to negative ME pressure.[2]

This classical dogma, however, was recently questioned by several studies; thus Buckingham et al[3] reported failure to evoke negative pressure in the MEs of dogs whose ETs were blocked, a situation in which air reentry into the ME obviously was prevented.

Two other studies of a different nature, by Bylander et al[4] and by Hergils and Magnuson,[5] found that early morning ME tympanometric pressure is higher than that measured later in the day. Oxygen is presumed to be absorbed at night just as during the day, yet swallowing is considered to occur less frequently then. Therefore, in the presence of continued O_2 absorption and decreased swallowing, one would expect decreased, not raised, morning ME pressure.

Other studies maintain that a negative ME aeration balance is secondary to air's escaping through the ET, rather than due to O_2 absorption into the bloodstream. This is the so-called sniff theory.[6] Cantekin et al[7] also regard the continuous absorption of gas from the ME as questionable.

Our study is an attempt to trace the fate of CO_2, O_2, nitrogen, and air in atelectatic MEs. It was prompted by the known fact that once gas is introduced into an ear with a retracted drum, the drum will inflate, but then tend to revert to its original retracted position.[8] We conceived that the speed or mode of disappearance of the gases introduced into the ME may indicate the route a gas takes when it leaves the ear. Should this occur abruptly and with equal speed for different types of gases, this would denote that the gases escaped through the ET. Conversely, if the gases disappear slowly and with a speed that is in accordance with the solubility coefficient of the particular gas used, this would indicate that the gases were absorbed into the bloodstream and body tissues.

MATERIAL AND METHODS

Thirteen patients (average age, 29.7 years; range, 15 to 69 years) with atelectatic ears or retraction (Fig 1) served as an experimental model. The atelectatic ears and retractions were of grades 1 through 3. No adhesive cases, ie, grade 4, were included.[9] Two patients (15.38%) presented atelectasis of the entire drum, and 11 (84.61%) presented atelectasis or retraction of parts of the drum. Six patients (46.15%) showed atelectasis grade 1, three (23.07%) showed atelectasis grade 2, and four (30.76%) showed atelectasis grade 3 (Fig 2).

Politzer bags were filled alternately with CO_2, O_2, air, or N_2. The gas concentration was checked periodically using pH/blood gas analyzer ABL3 produced by Radiometer Copenhagen. Our politzer bags contained the following average concentration of the gases: CO_2, 87%; O_2, 65%; and N_2, 96%.

Each patient was politzerized with CO_2, O_2, air, and N_2. Altogether, 90 such measurements were performed. At the time of politzerization the tympanic membrane (TM) was observed under the operating microscope by a second person (Fig 3). A

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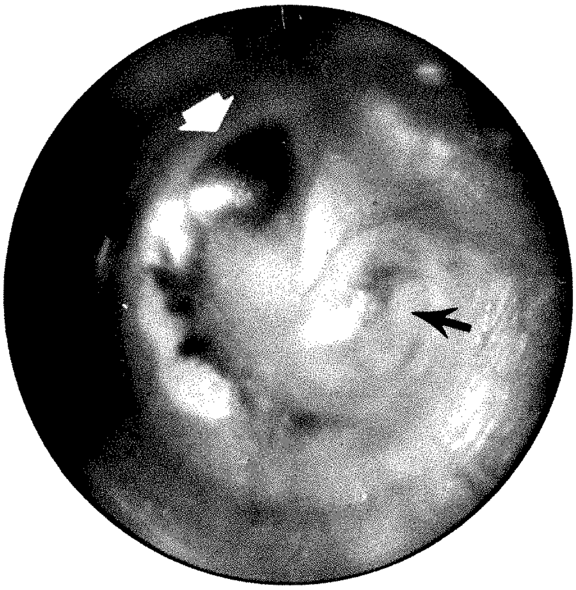

Fig 1. Atelectasis of most of tympanum. White arrow points to anterior region, where immediately behind it drum is atelectatic. Black arrow points to posterior region, where drum is seen collapsed over incus. Malleus is prominent between anterior and posterior parts of drum. Note tympanosclerotic deposits anteroinferiorly.

given ME was considered to be filled with the politzerized gas once its TM stretched and showed no more retraction (Fig 4).

After the introduction of the gas into the ME, observation of the TM continued nonstop until the TM returned to its previous retracted position. The time it took for the drum to return to its original state was taken as the time it took for the added gas to leave the ear.

RESULTS

Politzerization with any of the gases filled all examined ears without exception. In some ears this was achieved readily; others required several politzerizations.

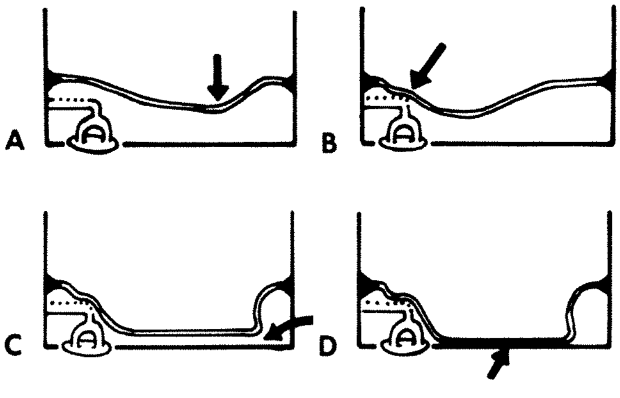

Fig 2. Atelectasis grades. A) Grade 1 shows slight retraction of drum. B) Grade 2 shows severe retraction and its progression until drum touches incus. C) In grade 3, true atelectasis, drum is retracted further, reaching promontory. D) In grade 4, adhesive otitis, adhesions bind drum to promontory.

Fig 3. Politzerization performed by physician while second physician observes tympanic membrane at same time.

Once a given gas was introduced into the middle ear, the TM immediately stretched to a nonretracted state. Now the drum usually looked as if it were in its usual place, ie, it reached its "zero point." Sometimes, however, outward ballooning occurred. This was noted especially in the previously retracted atrophic part of the drum (Fig 4).

Eventually, all stretched TMs returned to their original retracted position. The return of the TM to its original position was observed to take place in all instances very gradually, provided patients did not sniff, swallow, or perform a Toynbee maneuver.

When the retracted TM returned gradually to its original state, it did so at a speed that was different for each of the different gases. The difference in speed was proportionate to the diffusion coefficient of the particular gas. The average speed for the disappearance of the different gases was 5.37 minutes

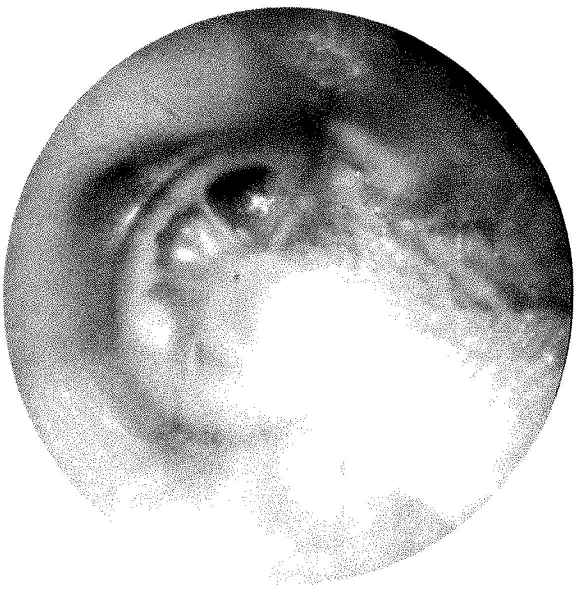

Fig 4. Atelectatic drum after politzeration. Same ear as in Fig 1. Note disappearance of atelectasis and ballooning of posterior part of drum.

(range, 1 to 15 minutes) for CO_2, 33.32 minutes (range, 14 to 90 minutes) for O_2, 53.37 minutes (range, 15 to 120 minutes) for air, and 104.76 minutes (range, 40 to 335 minutes) for N_2.

Once the TM was stretched or ballooned after politzerization, some patients could voluntarily make their TM return abruptly to its original retracted level. One patient (7.69%) could do it by swallowing, four patients (30.76%) by performing the Toynbee maneuver, and four patients (30.76%) by sniffing.

Two of our patients informed us that sniffing was for them a habit they performed to relieve an uncomfortable feeling in the ear. For these patients having the drum at zero point and not retracted equalled discomfort. The retracted steady state was for them a comfortable situation.

At least three patients were seen to be able to fill their atelectatic MEs with air, one through repeated swallowings, and two while yawning.

DISCUSSION

Gases added by politzerization to an ME having a retracted drum reverse the retraction immediately, but only for a short time. Reappearance of the retraction occurs relatively swiftly, and it may be logical to conclude that this happens concomitantly with disappearance of the added gases. Our experiments show that the gases may leave the ear through two alternative paths.

When the ET does not play an active role, the gas disappears gradually. The speed depends regularly on the type of gas used, indicating diffusion into the general circulation. Speed of gas diffusion through semipermeable membranes depends in general on its pressure difference on both sides of the membrane and on its solubility index.[10] Perfusion conditions may play a significant role as well.[11] Consequently, CO_2 is expected to pass through a membrane swiftly, N_2 relatively slowly, and O_2 and air in between. This is indeed how the gases behaved in our study. A previous study has demonstrated that gases may pass similarly by diffusion to and from the normal ME lining.[3] Our present study establishes, therefore, that in atelectatic ears, O_2, CO_2, and N_2 obey the same laws that govern gas diffusion through biologic semipermeable membranes as they do in normal ears.[1,3]

We also have observed that gas may disappear from the ME swiftly, escaping instantly as one "bolus" of gas during sniffing or swallowing. This alternative route was observed in a third of the examined ears, and confirms previous communications by Miller[12] and Magnuson.[13] The abruptness with which this happens indicates that the gas could only have disappeared via the ET.

Our direct observations show also that air can sometimes enter the atelectatic ME through the ET during swallowing or yawning. While we have observed this to occur in three patients, we believe that a more persistent tracing of air entry into atelectatic ears through the ET will show this to happen more often.

In an airplane, one can feel air leaving or entering the ME through the ET on ascent or descent. Zöllner,[14] Elner et al,[15] and Bluestone et al[16] measured experimentally the pressures under which this entrance and exit of air occurs. These are the inflation-deflation tests and their derivatives. Their findings show that the normal ME copes with positive and negative pressures in a nonuniform way, within a rather wide range of pressures, some individuals coping much more easily than others.

Gases thus have two pathways for entering or leaving the normal as well as the atelectatic ME. One is through diffusion into or out of the ME lining, the other is as a bolus of air going into or out of the ET. A balance between the intake and output will provide for a physiologic situation. A negative imbalance may bring about a pathologic situation such as is found in atelectasis or retraction pocket. A positive imbalance seldom presents itself as a clinical problem.

The classical notion that aeration imbalance of the ME stems from ET obstruction now seems to be an oversimplification; indeed, recent studies have shown that in secretory otitis media and chronic otitis media the ET lumen is not even narrowed.[17-23] It is not known what brings about a negative aeration balance in these conditions, but the data point toward a quantitative difference of aeration in ears with deficient aeration. The lesson learned from this study is that a model of this quantitative imbalance should take into consideration both entry and exit of gas through the ET as well as through the circulation.

There remains the opening question of this study, which seems to defy some of the basic rules governing the system: why didn't the dogs in the study by Buckingham et al[3] develop a negative pressure? Possibly this was because 2 hours was too short a time to do so. When Proud et al[24] obstructed the tube in cats, it took a longer time before a measurable negative pressure did develop.

As to the raised morning pressure found in tympanometry by Bylander et al[4] and Hergils and Magnuson,[5] this could be an expression of nocturnally raised CO_2[25,26] that diffused from the blood into the ME. Also, nocturnally reduced metabolism may reduce tissue O_2 and consequently reduce its diffusion from the ME into the circulation. One may also consider that during sleep, relaxation of the ET muscles facilitates air passage through the ET. While we have confirmed the existence of sniffing, its role in the pathogenesis of atelectasis is still an enigma. It would be interesting to learn whether patients with atelectasis sniff more than normal control subjects.

CONCLUSIONS

The ET in atelectatic ears is, as a rule, patent to airflow as produced by politzerization. Carbon dioxide, O_2, air, and N_2 introduced into atelectatic MEs are reabsorbed and the ME arrives at its prepolitzerized state within a relatively short time.

The speed of the gas exchange in atelectatic ears is in proportion to the diffusion rate and partial pressure of the particular gases present in the ME. Gas absorption in atelectatic ears provides for subatmospheric pressure, as evidenced by the final speedy retraction of their TMs after politzerization. After politzerization, retraction of the TM also can occur through sniffing (40%), the Toynbee maneuver (35%), or swallowing (10%). This occurs secondary to loss of a bolus of air through the ET.

We saw air enter and fill the atelectatic ME through the ET upon the patient's swallowing or yawning. Gas basically can pass to and from the atelectatic ear via the same routes as in normal ears, ie, through the ET or by diffusion. This does not mean, however, that air passes to and fro in atelectatic ears in the same quantities as it does in normal ears.

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OTOLOGIC HISTOPATHOLOGY OF FABRY'S DISEASE

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Fabry's disease is a rare progressive X-linked recessive disorder of glycosphingolipid metabolism. The accumulation of glycosphingolipids occurs in virtually all areas of the body, including the endothelial, perithelial, and smooth-muscle cells of blood vessels, the ganglion cells of the autonomic nervous system, and the glomeruli and tubules of the kidney. Although otologic symptoms have been described in these patients, to our knowledge there have been no temporal bone histopathologic reports. We describe the clinical histories, audiometric results, and temporal bone findings of two patients with this rare disorder. Both patients demonstrated a bilateral sloping sensorineural hearing loss audiometrically. Middle ear findings of seropurulent effusions and hyperplastic mucosa were seen in all four temporal bones. Strial and spiral ligament atrophy in all turns, and hair cell loss mainly in the basal turns, were also common findings. The number of spiral ganglion cells was reduced in all temporal bones; however, evidence of glycosphingolipid accumulation was not observed in the spiral ganglia.

KEY WORDS — Fabry's disease, glycosphingolipids, temporal bone histopathology.

INTRODUCTION

Angiokeratoma corporis diffusum universale, more commonly known as Fabry's disease, is a rare disorder transmitted by an X-linked recessive gene. This systemic disorder of glycosphingolipid metabolism results from the progressive accumulation of galactosylgalactosylglucosyl ceramide in most tissues of the body.[1] This accumulation is the result of the absence of enzymatic trihexosyl ceramide galactosyl hydrolase activity.[2] The disease usually becomes evident during childhood and adolescence, the hemizygous males being affected more severely than the heterozygous females.[3] The clinical symptoms include pain in the extremities, punctate skin lesions, corneal opacities, and peripheral edema. Involvement of the cardiovascular, renal, and central nervous systems occurs later, with death usually resulting from these complications in adult life.[1,4-6]

Sweeley and Klionsky[7] classified Fabry's disease as a sphingolipidosis following the isolation and characterization of two neutral glycosphingolipids, a trihexosyl ceramide, (galactosylgalactosylglucosyl ceramide) and a dihexosyl ceramide (galactosylgalactosyl ceramide). These neutral glycosphingolipids are deposited in all areas of the body, occurring predominantly in the endothelial, perithelial, and smooth-muscle cells of blood vessels and to a lesser extent in histiocytic and reticular cells.[1,8] Lipid deposits are also prominent in epithelial cells of the cornea and the glomeruli and tubules of the kidney, in muscle fibers of the heart, in ganglion cells of the autonomic nervous system, and in peripheral Schwann cells.[9]

Because of the systemic nature of the disease and the fact that deposits of glycosphingolipid occur in vessels and ganglion cells, it is not surprising to find that some patients demonstrate otologic symptoms. Staughton and Clendenning[10] described a patient with Fabry's disease who complained of intermittent positional vertigo accompanied by nausea and vomiting. This patient also had a history of tinnitus and hearing loss. Consultants suggested that the vertigo was secondary to hydrops of the labyrinth.

De Groot[11] described seven patients with Fabry's disease. Two of these patients (one hemizygous and one heterozygous for Fabry's) complained of dizziness. The hemizygote also suffered progressive deafness and tinnitus and had Meniere's syndrome. In the same paper, De Groot reviewed otologic symptoms in 16 cases described previously by others. Seven of these patients had dizziness, two had vertigo, two tinnitus, and one deafness. Bird and Lagunoff[12] reported a brother and two sisters with Fabry's disease, each experiencing episodic vertigo and nystagmus. The brother and one of the sisters also demonstrated hearing loss and tinnitus. Meyerhoff and Liston[13] reported that approximately one third of the patients with Fabry's disease seen at the University of Minnesota Otolaryngology Clinics had a sensorineural hearing loss in the high frequencies. Although patients with Fabry's disease and related otologic dysfunction have been described clinically, to our knowledge this is the first report to describe the histopathologic changes that occur in such patients.

MATERIALS AND METHODS

Temporal bones were harvested at autopsy with an oscillating Schuknecht bone-plug cutter and were fixed in 10% formalin. Following decalcifica-

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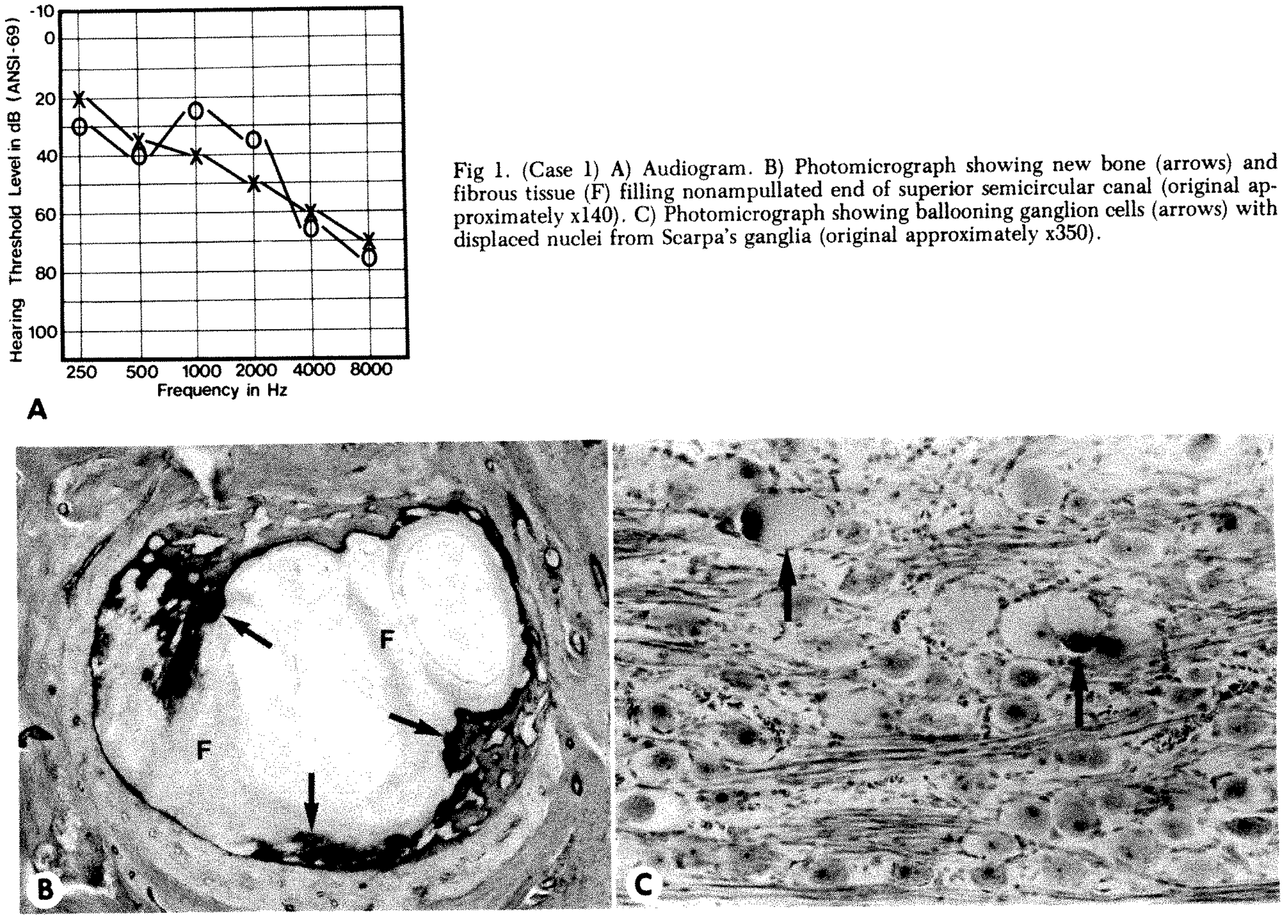


Fig 1. (Case 1) A) Audiogram. B) Photomicrograph showing new bone (arrows) and fibrous tissue (F) filling nonampullated end of superior semicircular canal (original approximately x140). C) Photomicrograph showing ballooning ganglion cells (arrows) with displaced nuclei from Scarpa's ganglia (original approximately x350).

tion, dehydration, and embedding in celloidin, the specimens were sectioned horizontally at a thickness of 20 μm, and every tenth section was stained with hematoxylin and eosin for histologic study. Histopathologic descriptions of left and right temporal bones are combined in each of the two cases presented because of the bilateral symmetry of the pathologic findings in each case.

RESULTS

CASE 1

Medical History. This was the 12th and final admission for a 48-year-old man with Fabry's disease. Diagnosis was made 7 years previously, following the death of his brother, who died at the age of 47 from pulmonary involvement secondary to Fabry's disease. His other brother subsequently died from the same secondary complication at the age of 53. The patient's 73-year-old mother was found to be the carrier. His mother complained of edema in her legs and of bilaterally diminished hearing for several years. His daughter was also diagnosed as a carrier, and five first cousins between the ages of 27 and 35 died of renal disease.

The patient's first admittance to the University's hospital was at age 47 for evaluation for a kidney transplant. The patient had a history of decreased sweating and of intermittent pain in the extremities. Physical examination revealed angioma and corneal whorling. A sloping bilateral sensorineural hearing loss was found at audiologic evaluation (Fig 1A).

The patient underwent a splenectomy and right nephrectomy followed by a right double cadaver kidney transplant. Postoperative complications of rejection and pneumonia, sepsis, and cardiac arrhythmia followed transplantation. The rejection episode was unresponsive to immunosuppressive and irradiation therapy, necessitating a left cadaver kidney transplant. The second transplant rejection was responsive to irradiation and immunosuppressive therapy; however, postoperative pneumonia developed, complicated by the presence of multiple organisms. The patient was unresponsive to treatment with multiple antibiotics and antifungal agents and died a few days later.

The neuropathologic report described neuronal lipidosis involving the hypothalamus, amygdala, temporal cortex, and dorsal vagal nucleus and intermediolateral nucleus of the spinal cord, as well as lipidosis of the autonomic and somatic ganglia and perineural sheaths. Lipidosis of the blood vessels was described as diffuse.

Findings in Temporal Bone. The mucoperiosteum of the middle ear and mastoid was thickened by fibrosis, sparsely infiltrated with chronic inflammatory cells, and covered by hyperplastic squamous epithelium. Within the subepithelial space, small cytoplasmic vacuoles could be seen in vascular endothelium and in occasional fibroblasts. Mild focal areas of seropurulent effusion were observed throughout the middle ear cleft. The ossicles appeared normal, with the exception of hyperostosis of the stapedial crura.

Within the first genu of the facial nerve, the ganglion cells were swollen, with nuclei displaced eccentrically and undergoing karyolysis. This ballooning karyolytic appearance was also evident in the ganglia of the greater superficial petrosal nerve (GSPN). Geniculate and GSPN ganglia in the right ear were not available for study.

The organ of Corti was essentially normal. The stria vascularis was atrophic in each turn, as was the spiral ligament. Atrophy of the spiral ligament was most pronounced in the middle and apical coils. The remaining stroma of the spiral ligament contained numerous fibroblasts whose cytoplasm appeared vacuolized and distended. This cytoplasmic vacuolization also was observed in endothelial cells lining the vessels of the vas spiralis and proximal modiolus. Cells in the spiral ganglion were morphologically normal but were moderately decreased in number in the basal turn. Mild cochlear hydrops was noted in the apical turn and focal *Candida* sp was observed in the perilymphatic space of the cochlear hook.

The saccule, utricle, and vestibule revealed no morphologic abnormalities; however, in the nonampullated end of the superior semicircular canal, new bone filled the perilymphatic space (Fig 1B). Scarpa's ganglia (right ear) exhibited ballooning ganglion cells consistent with those neurons previously mentioned (Fig 1C).

CASE 2

Medical History. A 59-year-old man was diagnosed as having Fabry's disease at the age of 49 on a routine admission for the repair of an inguinal hernia. The patient had a history of edema in his legs since age 27, of angioma since about age 9, and of decreased sweating for many years. His brother, who also had these symptoms, died of renal failure at age 45; however, no autopsy was performed to confirm a diagnosis of Fabry's disease. The patient's mother died at age 76 of kidney disease. It was felt that she probably had the subclinical form of the disease, because of the presence of corneal dystrophy and of lipid-filled vacuoles in the epithelial cells of her renal glomeruli and myocardium.

The patient was admitted to the University of Minnesota Hospital over 20 times for edema in the leg, pneumonia, chronic brain syndrome, and two myocardial infarctions during the 10 years between the diagnosis of Fabry's disease at age 49 and his death at age 59. He complained of a decrease in hearing since age 47. An audiologic evaluation of pure tone air and bone conduction thresholds at age 50 revealed a sloping sensorineural hearing loss bilaterally (Fig 2A).

His final admission to the hospital was due to arrhythmia and edema of the legs. The patient's condition progressively deteriorated and he died of cardiopulmonary arrest 3 days after admission.

The neuropathologic report at autopsy described neuronal lipidosis involving the hypothalamus, amygdala, temporal cortex, and dorsal vagal nucleus and intermediolateral nucleus of the spinal cord, as well as of the autonomic and somatic ganglia and perineural sheaths. Lipidosis of the blood vessels was described as diffuse.

Findings in Temporal Bone. The mucosa of the middle ear was thickened, hyperemic, and diffusely lined with hyperplastic epithelium contiguous with a scarred tympanic membrane. Focal areas of seropurulent effusion were noted throughout the tympanum bilaterally. This was accompanied in the left ear by vascularized fibrous adhesions in the mastoid. Occasional small vessels in the mucoperiosteum displayed cytoplasmic foaminess of the endothelial and smooth muscle cells.

Ganglion cells exhibited a ballooning appearance with granulating nuclei in the tympanic ganglia (Jacobson's nerve), Scarpa's ganglia, GSPN ganglia, and semilunar (gasserian) ganglia. Semilunar and GSPN ganglia in the left ear were not available for study. Cells in the spiral ganglion revealed no morphologic abnormalities; however, populations were profoundly decreased in all cochlear turns.

Examination of the organ of Corti revealed moderate loss of outer hair cells in each turn and an often collapsed tectorial membrane. This finding was more severe in the right ear. Endothelial cells lining the internal auditory artery, modiolar vessels, and vas spirale appeared distorted because of their foamy vacuolized cytoplasm. The stria vascularis was markedly atrophic in all cochlear turns, and the spiral ligament demonstrated profound acellularity in the middle and apical coils (Fig 2B,C). Vestibular structures were normal with the exception of mild hemorrhage within the saccule of the left ear.

DISCUSSION

Fabry's disease, like the various other lipid storage diseases (Gaucher's disease, Niemann-Pick disease, Krabbe's disease, Tay-Sachs disease, and generalized gangliosidosis), is characterized by the accumulation of lipids in most tissues of the body. Although it would not seem surprising to find hearing problems in patients with some form of sphingo-

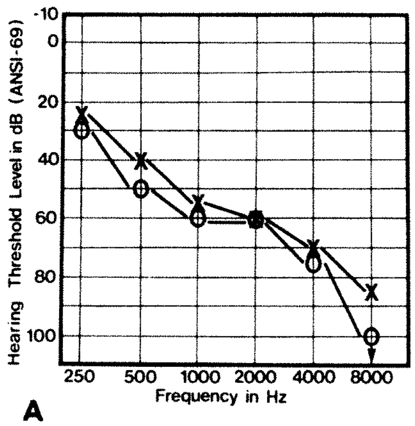
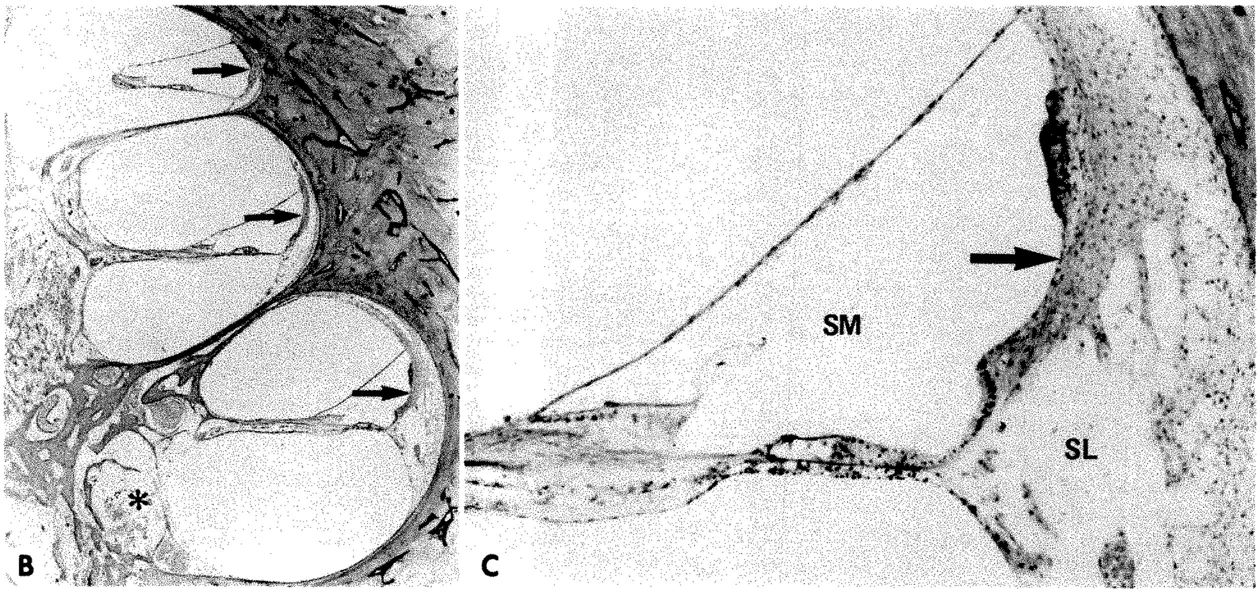


Fig 2. (Case 2) A) Audiogram. B) Photomicrograph showing atrophy of stria vascularis (arrows) and spiral ligament (original approximately x35). Note decrease in ganglion cell population of basal turn(✱). C) Higher magnification of first turn (original approximately x140). Note atrophy of stria (arrow) and spiral ligament (SL) and loss of outer hair cells. SM — scala media.



lipidosis, evidence concerning the true incidence and underlying pathophysiology of any association has been lacking. We have reviewed the clinical reports of hearing loss in patients with Fabry's disease and have examined the temporal bones and clinical histories of two of these patients, to determine those pathophysiologic changes that occur in the ear because of the systemic nature of the disease. Because Fabry's disease affects virtually every tissue of the body (kidneys, heart, blood vessels, ganglia, etc), one would expect that the histopathologic findings would vary greatly between patients. We were surprised to find both the audiometric findings and the histopathologic changes in the temporal bones in the two cases we examined to be quite similar.

Audiometric findings for both patients revealed bilateral sloping sensorineural hearing losses, and neuropathologic findings at autopsy were identical. Histopathologic findings of bilateral hyperplastic mucosa of the middle ear and seropurulent effusions, evidence of glycosphingolipid accumulation in the ear in vascular endothelial cells and in various ganglion cells, and atrophy of the stria and spiral ligament in all cochlear turns was observed in both cases. The strial and ligamentous atrophy in all four temporal bones, the loss of hair cells in one case, and formation of new bone in the superior semicircular canal of the other may be due to vascular obstruction. Accumulation of glycosphingolipids in patients with Fabry's disease is known to occur predominantly in the endothelial, perithelial, and smooth muscle cells of blood vessels,[1-8] and we observed evidence of accumulation throughout the vasculature of the middle and inner ears.

Renal tubules and the stria vascularis have been shown to demonstrate many anatomic and enzymatic similarities.[14,15] The most dramatic accumulation of glycosphingolipid ceramide trihexoside in Fabry's disease is in the renal glomeruli and tubules.[9] It may be that the strial atrophy observed in the four temporal bones examined was due, at least in part, to similarly high levels of accumulation within the stria. It is also possible that the strial damage was not caused by a direct effect on the stria itself, but by a secondary effect caused by damage to the kidney. Damage to the kidney in and of itself has been associated with hearing loss.[16-18]

We believe that the histopathologic changes that result in dysfunction in the ear in patients with Fabry's disease are due to both a direct effect on the ear and an indirect effect resulting from damage to other tissues. The direct effect is caused by accumulation of glycosphingolipids within the stria vascularis and ganglion cells, and the indirect effect by accumulation of glycosphingolipids within the temporal cortex, renal glomeruli and tubules, and endothelial and smooth muscle cells. Previous clinical studies of patients with Fabry's disease and this histopathologic study indicate that hearing loss is a common problem in patients with Fabry's disease and in other lipid storage diseases as well.[19] Hearing loss also has been reported in patients with abnormalities of bilirubin metabolism,[20] glucose metabolism,[21] protein metabolism,[13] and mucopolysaccharide metabolism.[22] We believe that hearing problems can occur in patients with storage diseases, and the physician should be aware of the possibility of an otologic component in these patients.

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EVALUATION OF PURE TONE AND SPEECH DISCRIMINATION CHANGES IN USHER'S SYNDROME

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Twenty-three adult patients with type 2 Usher's syndrome were evaluated for changes in pure tone average (PTA), discrete frequency thresholds, and speech discrimination scores. These patients were evaluated over a period of 5 years on the average (range, 2 to 9 years). Analyses of ear data showed a $<$10-dB change on PTA and discrete frequency thresholds for most ears. Of the ears that showed a threshold change $\geq$10 dB, statistical significance was reached only at 4,000 Hz ($p<.01$), where 11 ears representing ten patients dropped 10 to 15 dB. Speech discrimination data showed a $\geq$16% change in score for 18 ears (12 patients). Sixteen of those ears (ten patients) had a 16% to 52% decrease in score ($p<.01$), and the remaining two ears (two patients) showed a 20% and 30% improvement in score. These findings document little or no change in pure tone sensitivity for type 2 Usher's syndrome patients, but demonstrate the importance of monitoring their speech discrimination performance.

KEY WORDS — retinitis pigmentosa, sensorineural hearing loss, Usher's syndrome.

INTRODUCTION

Usher's syndrome combines congenital sensorineural hearing loss with retinitis pigmentosa, a progressive night blindness disorder. The syndrome was first recognized by von Graefe[1] in 1858, but was described more extensively by Usher in 1914.[2] As the leading cause of combined deafness and blindness in the United States, Usher's syndrome has a prevalence rate among the congenitally deaf population ranging from 3% to 6%.[3]

Clinical expressivity is variable and hereditary transmission is autosomal recessive.[4,5] In addition to the auditory and visual impairments, some investigators also have noted associated neurologic or psychiatric disorders.[3,6] Merin et al[7] and Fishman et al[8] have proposed classification of Usher's syndrome into clinically distinct subtypes based on differences in expressivity.

The sensorineural hearing loss associated with Usher's syndrome usually has been described in terms of pure tone loss. The degree of loss may range from mild to profound, and the audiometric configuration typically shows the greatest loss at high frequencies.[3,5,6,9] Some patients have shown progressive pure tone loss,[6,7,10-13] while others have not.[12,14,15] Methods have varied, with some investigators using audiometry and others relying on patients' reports to judge progressive hearing impairment. Although infrequently reported, most measurable speech discrimination scores have fallen between 40% and 74%.[5,10,12] Bekesy, SISI, and tone decay results obtained on a few patients have been consistent with cochlear disease.[5,12]

Given the progressive nature of the visual handicap associated with Usher's syndrome, it is surprising that the question of potentially progressive hearing impairment has not received greater attention. Therefore, the purpose of this investigation was to identify possible changes over time in the pure tone sensitivity and speech discrimination performance of Usher's patients.

METHODS

Audiometric data were analyzed for 23 patients with type 2 Usher's syndrome, as described by Fishman et al.[8] Type 2 Usher's patients demonstrate moderate to profound hearing loss, intelligible speech, variable visual field loss from normal to marked, variable central vision from 20/20 to 20/200 or less, onset of nyctalopia (ie, night blindness) during the second or third decade, variably abnormal electroretinography results, and a vestibular response to caloric stimulation. The pedigrees obtained for these patients and the results of segregation analyses conducted by a geneticist were consistent with autosomal recessive inheritance, as anticipated for Usher's syndrome.[16] The patients represented 20 families and included 14 males and nine females.

Results were compared from two audiometric evaluations. Ages at the time of the first hearing test ranged from 16 to 52 years (mean age, 33 years), and ages at the time of the follow-up test ranged from 20 to 57 years (mean age, 39 years). The time interval between tests ranged from 2 years (one patient) to over 9 years (four patients), with a mean

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TABLE 1. AUDIOMETRIC AND SPEECH DISCRIMINATION RESULTS FOR 23 USHER'S SYNDROME PATIENTS

Pt No.	Age at Baseline (yr)	Intertest Interval (yr-mo)	Test No.	Right Ear (Hz)							Right Ear, Speech Discrimination (%)	Left Ear (Hz)							Left Ear, Speech Discrimination (%)
				PTA (dB)	250 (dB)	500 (dB)	1,000 (dB)	2,000 (dB)	4,000 (dB)	8,000 (dB)		PTA (dB)	250 (dB)	500 (dB)	1,000 (dB)	2,000 (dB)	4,000 (dB)	8,000 (dB)	
1	21	4-10	1	48	20	40	50	55	50	55	84	47	25	40	50	50	50	65	84
			2	48	25	40	55	50	55	65	80	50	30	45	50	55	60	65	80
2	36	9-3	1	48	15	35	50	60	60	70	90	48	15	35	55	55	60	65	92
			2	55	25	40	60	65	65	70	68	48	25	35	50	60	60	65	92
3	36	4-4	1	53	15	30	60	70	65	70	88	48	15	25	55	65	60	70	88
			2	55	10	30	65	70	70	85	84	55	15	35	60	70	70	85	64
4	42	5-2	1	55	30	40	60	65	65	70	84	48	35	40	50	55	65	75	84
			2	52	35	35	60	60	75	80	84	50	30	35	60	55	75	80	96
5	24	4-6	1	50	45	45	50	55	60	60	88	50	40	45	55	50	55	55	84
			2	57	40	55	55	60	70	65	80	55	35	50	55	60	60	65	80
6	48	4-0	1	52	45	50	45	60	75	90	78	55	45	45	55	65	70	75	72
			2	55	40	45	55	65	85	90	50	52	45	45	50	60	70	80	52
7	16	4-5	1	57	35	40	60	70	65	50	88	52	30	40	45	70	60	50	88
			2	55	30	45	55	65	60	55	92	53	30	40	55	65	60	60	88
8	22	9-4	1	53	35	40	55	65	60	75	94	55	25	40	60	65	70	75	96
			2	55	40	45	55	65	60	60	80	48	25	40	50	55	60	60	96
9	33	4-5	1	58	50	55	55	65	70	60	72	53	40	45	55	60	60	60	68
			2	58	45	45	60	70	65	70	58	52	35	40	55	60	65	70	68
10	46	9-4	1	55	25	50	55	60	55	80	92	55	35	50	60	55	55	80	92
			2	53	40	50	55	55	55	65	72	53	35	50	55	55	60	70	92
11	37	6-5	1	62	25	50	70	65	70	90	82	57	35	50	60	60	70	90	92
			2	60	35	55	60	65	70	90	96	55	30	50	55	60	65	90	96
12	33	6-2	1	58	45	50	60	65	70	80	76	62	55	55	65	65	65	75	64
			2	62	55	65	60	60	75	85	86	67	60	65	70	65	80	85	84
13	26	4-8	1	62	50	55	65	65	60	75	88	58	55	55	60	60	60	70	88
			2	60	45	50	65	65	65	75	72	52	40	45	50	60	70	80	72
14	36	4-4	1	60	40	55	60	65	80	70	84	63	35	55	65	70	70	65	88
			2	63	35	50	65	75	75	90	44	72	55	70	75	70	70	80	36
15	37	2-4	1	68	40	50	75	80	85	100	64	60	45	45	65	70	80	90	84
			2	63	35	40	75	75	85	90	72	55	45	45	55	65	75	90	84
16	32	5-4	1	63	35	50	65	75	85	NR	DNT	63	40	50	65	75	90	NR	DNT
			2	63	35	50	65	75	95	90	80	63	35	50	65	75	95	90	88
17	27	4-9	1	67	45	55	70	75	70	70	84	63	50	55	60	75	70	65	88
			2	70	50	65	70	75	65	80	42	60	45	50	50	80	65	80	68
18	21	9-5	1	63	50	60	70	60	60	60	DNT	68	55	65	75	65	70	65	DNT
			2	57	40	50	65	55	60	65	84	70	55	70	80	60	65	60	68
19	52	5-8	1	65	35	55	70	70	80	90	64	67	40	60	70	70	80	90	76
			2	65	35	55	70	70	90	90	72	67	40	60	70	70	80	90	56
20	40	5-7	1	72	20	50	80	85	110	90	72	73	40	45	70	105	110	90	80
			2	78	40	60	80	95	105	95	48	87	55	70	85	105	105	90	32
21	33	4-5	1	90	80	80	100	90	85	70	40	92	70	80	100	95	85	75	56
			2	97	65	80	110	100	100	80	12	95	70	85	100	100	85	75	24
22	24	2-10	1	97	55	85	95	110	NR	NR	8	95	60	90	100	95	90	NR	36
			2	98	60	85	100	110	110	90	38	97	55	90	100	100	85	90	28
23	43	4-4	1	102	75	100	110	95	110	90	DNT	108	90	110	110	105	110	90	DNT
			2	102	75	95	110	100	110	90	DNT	110	90	110	110	110	110	90	DNT

Test 1 is baseline; test 2 is follow-up.

PTA — pure tone average, NR — no response, DNT — did not test.

TABLE 2. NUMBER OF EARS SHOWING THRESHOLD CHANGE BETWEEN FIRST AND SECOND TESTS

Amount of Change	*PTA (n = 46)*	*250 Hz (n = 46)*	*500 Hz (n = 46)*	*1,000 Hz (n = 46)*	*2,000 Hz (n = 46)*	*4,000 Hz (n = 45)*	*8,000 Hz (n = 42)*
15 dB improvement	—	2	—	—	—	—	3
10 dB improvement	—	1	4	5	1	1	2
< 10 dB change	45	35	34	34	41	33	22
10 dB decrease	—	4	5	6	4	9	10
15 dB decrease	1	2	2	1	—	2	4
20 dB decrease	—	2	—	—	—	—	1
25 dB decrease	—	—	1	—	—	—	—

intertest interval of 5 years 6 months. Age and intertest interval for each patient are presented in Table 1.

Pure tone thresholds were obtained at octave frequencies from 250 to 8,000 Hz for air conduction and from 500 to 4,000 Hz for bone conduction. All threshold testing used 5-dB intensity increments. Speech discrimination was measured at the most comfortable loudness level, which for the majority of patients was a sensation level (SL) between 25 and 45 dB. For patients with reduced loudness tolerance, 15 to 20 dB SL was used. For most of the baseline evaluations, speech discrimination was measured with CID W-22[17] word lists delivered by monitored live voice. At the time of the follow-up tests the usual procedure was to use tape recorded NU-6 lists[18] (Auditec of St Louis test tapes). Approximately one third of the speech discrimination scores were based on full-list presentations (50 words), and the remaining scores were based on half-lists (25 words). Audiometric equipment was calibrated to ANSI (1969)[19] standards, and all tests were conducted in sound-treated test booths.

RESULTS

Table 1 presents the audiometric and speech discrimination data obtained for each patient at the baseline test (test 1) and the follow-up test (test 2). On the basis of the three-frequency pure tone average (PTA) at 500, 1,000, and 2,000 Hz, 19 patients had moderate loss bilaterally (41 to 70 dB HL), one patient had severe loss bilaterally (71 to 90 dB HL), and three patients had profound loss bilaterally (>90 dB HL). Results of the baseline and follow-up tests were evaluated for possible changes in PTA, discrete frequency threshold, and speech discrimination score.

As shown in Table 1, the changes in pure tone response levels between the two tests were generally small. Most threshold changes were <10 dB, although a number of changes ranging from 10 to 25 dB also occurred. Table 2 summarizes these changes by presenting the number of ears showing various amounts of threshold change. These data are presented in reference to ears rather than patients because some individuals showed threshold changes that varied across frequencies or between ears. For example, patient 20 (Table 1) had a 15-dB change in threshold at 1,000 Hz in the left ear and no change in the right ear at that frequency.

The PTA data in Table 2 show <10 dB change between tests for 45 ears, and a 15-dB drop in PTA for only one ear. Table 2 shows that most ears also had <10 dB change in discrete frequency thresholds. Some ears, however, showed a larger change in threshold at specific frequencies, ranging from a 15-dB improvement to a 25-dB decrease. It is known that small variations in threshold may be due to test-related factors such as earphone placement, test instructions, and patient variability.[20] In order to evaluate whether the changes observed for these patients were random or whether significant trends were present in the data, the Wilcoxon matched-pairs signed ranks test was used to analyze the direction and magnitude of threshold changes that were ≥10 dB. The statistical results, shown in Table 3, were significant only at 4,000 Hz ($p < .01$). Of the ears demonstrating a change in threshold at 4,000 Hz, a significant number showed a drop in threshold. Table 2 shows that nine ears dropped 10 dB and two ears dropped 15 dB. These ear data represented ten of the 23 patients (43%), tested over a 4- to 6-year period (mean, 4 years 11 months). The drop in threshold at 4,000 Hz was unilateral for nine patients and bilateral for one patient.

The final measure, speech discrimination score, was evaluated for 20 patients. Separate ear scores also were used in the analysis of these results, since some patients showed differences between ears (eg, patient 10). Speech discrimination scores at baseline ranged from 8% to 96% correct (mean, 78%), while scores at the follow-up test ranged from 12% to 96% correct (mean, 68%). Although the group data were similar between the first and second tests, some individual ears showed large differences, as shown in Table 1. The number of ears showing various amounts of change in discrimination score are summarized in Table 4. Only changes ≥16% were considered in the statistical analysis because of the test-retest variability associated with speech discrimination scores[21] and because a similar criterion has been recommended for the evaluation of speech discrimination change in Meniere's disease.[22] With this criterion, 16 ears showed a decrease in discrimination score ranging from 16% to 52%, and two ears showed a 20% and 30% improvement in score, respectively. Of these 18 ears with a

TABLE 3. WILCOXON MATCHED-PAIRS SIGNED-RANKS ANALYSES FOR EARS SHOWING THRESHOLD CHANGE BETWEEN FIRST AND SECOND TESTS

Frequency (Hz)	*No. of Ears*	*No. in Which Threshold Dropped at Retest*	*Wilcoxon T Value*
250	11	8	18.0
500	12	8	20.0
1,000	12	7	30.0
2,000	5	4	3.0
4,000	12	11	5.5*
8,000	20	15	61.0

*Statistically significant ($p < .01$, two-tailed).

change in discrimination score, the number showing a drop in score was significant (Wilcoxon matched-pairs signed-ranks test, $T = 17.0$, $p < .01$, $N = 18$). The 16 ears with decreased scores represented ten of the 20 patients (50%). Speech discrimination scores decreased bilaterally for six patients and unilaterally for four patients.

Several patient-related factors that might affect speech discrimination results were evaluated, including age, intertest interval, discrimination score on the baseline test, and 4,000-Hz threshold. The average age of the ten patients showing a $\geq 16\%$ decrease in speech discrimination score was 38 years, and their mean intertest interval was 5 years 8 months. The average age of the remaining patients was 29 years, and their mean intertest interval was 5 years. Thus, the patients with decreased speech discrimination were slightly older than the other patients, and they had been tested at a slightly longer interval. No significant relation was found between speech discrimination score on the baseline test and the amount of change found at retest (Pearson $r = -.26$, $p > .05$). Finally, only four ears (four patients) with decreased discrimination also showed a drop at 4,000 Hz.

DISCUSSION

Previous studies concerned with possible progressive hearing loss among Usher's syndrome patients have been based on findings for one or only a few subjects and primarily have considered only pure tone findings. Some reports have described marked deterioration in hearing, while others have described stable auditory function.

Beatty et al[10] reported results for a 29-year-old patient whose pure tone loss progressed over a 6-year period from severe-profound to profound. During the same period, speech discrimination scores dropped from 44% and 48% to no response in either ear. Goode et al[12] described a 47-year-old patient in whom pure tone responses showed no change over a 3-year period. However, speech discrimination dropped in one ear from 60% to 8%, and in the other ear from 48% to 36%. Although earlier audiograms were not presented, the patient reported gradual progression of hearing loss until age 35. Karp and Santore[13] reported three young adults tested over a 10- to 13-year period in whom pure tone results worsened.

TABLE 4. NUMBER OF EARS SHOWING CHANGE IN SPEECH DISCRIMINATION SCORE BETWEEN FIRST AND SECOND TESTS

Amount of Change	*No. of Ears (n = 40)*
20%-30% improvement	2
< 16% change	22
16%-26% decrease	9
28%-38% decrease	3
40%-52% decrease	4

In contrast, no change in pure tone responses was reported by Goode et al[12] for three children tested at various intervals between ages 7 and 15 years. McGovern[15] evaluated four brothers over a 20-year interval and stated that the pure tone audiograms were "essentially the same." De Haas et al[14] also reported no appreciable change in pure tone audiogram for one patient tested at ages 10 and 20 years, or for three patients tested during their mid-20s and again in their mid-30s.

Our data, based on evaluations of adult Usher's syndrome patients, showed generally small changes in pure tone thresholds (ie, < 10 dB) measured over an average period of 5 years. Threshold change reached statistical significance only at 4,000 Hz, where 11 ears, or ten patients (43%), showed a 10- to 15-dB drop in threshold. Despite this statistical significance, the amount of threshold change was clinically small.

Larger changes were found in speech discrimination scores, in that 16 ears (ten patients) had decreases in score ranging from 16% to 52%. While these changes were sizable, their clinical significance has yet to be better defined. Speech discrimination test procedures varied in the present study, in that live-voice CID lists usually were used during the first evaluations and tape-recorded NU-6 lists generally were used during the second evaluations. While the intelligibility functions of CID and NU-6 lists may be similar, at least for normal listeners,[23] other factors such as speaker and carrier phrase can contribute to variability in subject performance.[24,25] Nonetheless, the magnitude of the changes demonstrates the importance of monitoring speech discrimination in Usher's syndrome patients and suggests that for some patients speech discrimination performance may change markedly even when there has been little or no change in pure tone sensitivity.

As we continue to follow these patients, pure tone thresholds and speech discrimination scores will be monitored uniformly over a longer period of time. In view of the progressive nature of the visual disorder involved with Usher's syndrome, an understanding of the nature and natural history of the auditory impairment is vital to appropriate patient counseling and rehabilitation.

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EFFECT OF TEMPERATURE ON FIXATION SUPPRESSION ABILITY IN NORMAL SUBJECTS: THE NEED FOR TEMPERATURE- AND AGE-DEPENDENT NORMAL VALUES

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The purpose of the present investigation was to determine whether temperature has an effect on the magnitude of fixation suppression (FS) in normal subjects. The subject population consisted of 23 patients ranging in age from 35 to 74 years (mean age of 58.13 years). Each subject received a conventional vestibulometric test battery that included bithermal caloric testing and the FS test. The warm caloric media elicited larger nystagmus slow-phase eye velocities than the cool caloric media. Following warm caloric irrigations, FS was significantly smaller than that following cool caloric irrigations. It also worsened as a function of subject age. The results suggest that separate normal upper limits for FS need to be determined for cool and warm caloric testing. They also suggest that FS ability may decrease with age.

KEY WORDS — electronystagmography, fixation suppression, vestibular response.

INTRODUCTION

The electrodiagnostic evaluation of patients who are vertiginous has changed little during the 45 years that have passed since Fitzgerald and Hallpike[1] introduced the alternate binaural bithermal water caloric test. The improvements in the vestibulometric test battery have included the development of 1) electro-oculographic (EOG) instrumentation, 2) methods to improve the test/retest reliability of the vestibulometric test battery,[2] and 3) techniques to evaluate the vestibulospinal and spinovestibular pathways.[3] One other improvement in the vestibulometric examination was the development of a method to evaluate the integrity of the neuronal pathways between the vestibulocerebellum (ie, the flocculus) and the mesencephalic visual centers and between the vestibulocerebellum and the vestibular nuclei.[4-6] This has been termed the fixation suppression (FS) test.

The FS test normally is conducted midway through the caloric examination. The patients are requested to open their eyes and fixate on a centrally positioned target. Normally the caloric nystagmus diminishes in intensity following visual fixation. The amount that the caloric nystagmus velocity attenuates in normal individuals following visual fixation varies by report between 50% and 70% independent of the stimulus temperature.[7-10] The FS phenomenon has been demonstrated for rotational stimulation.[11] The potentiation of vestibular nystagmus through fixation appears to be coordinated within the vestibulocerebellum (flocculus). There has been a great deal of controversy in recent years as to whether the pursuit visual subsystem is responsible for this function or whether FS is an independent function that shares common pathways with the pursuit system but is functionally autonomous. Convincing arguments have been raised in support of each viewpoint.[12-16]

It is surprising that so little is known about the effect that specific stimulus variables have on FS. For instance, there has been no prior investigation into whether the temperature of the caloric stimulus affects the degree of FS. It is now well accepted that warm (44°C) water caloric stimuli elicit stronger slow-phase eye (nystagmus) velocities (SPEV) than cool (30°C) caloric stimuli.[17] It is also known that the pursuit system can follow smoothly moving targets with a gain of 1.0 for targets that move from 30°/s to 60°/s. As this upper limit is exceeded the target slips off the retina and the smooth eye movements are replaced by catch-up saccades. The upper limit for smooth pursuit gain is affected by the subject age. Thus, older individuals are less able to follow pursuit targets with a gain of 1.0 as the target velocities exceed 5°/s.[18,19] It would follow that if the smooth pursuit pathway or some of the components of the pursuit pathway subserve FS, then the greater the prefixation caloric SPEV the poorer the FS. It has been reported[20] that no relationship was found between nystagmus velocity and FS ability. However, the subject sample in that investigation was extremely small ($n = 12$) and the statistical analysis techniques were not reported. The purpose of the present investigation was to determine whether the magnitude of the prefixation nystagmus velocity has an effect on the postfixation suppression ability. The finding of significant temperature-dependent differences in FS would suggest that temperature-dependent norms for FS should be determined to increase the sensitivity of this test.

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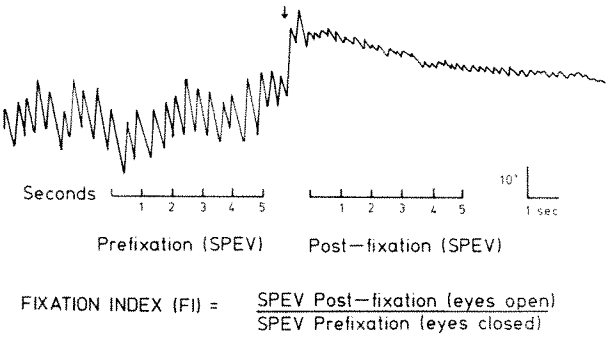

Fig 1. Electronystagmogram and depiction of method by which fixation index was calculated in present investigation (see text). SPEV — slow-phase eye velocity.

METHODS

The subjects were 23 patients ranging in age from 35 to 74 years of age (22 men and one woman with a mean age of 58.13 ± 11.15 years) who were referred for diagnostic electronystagmography (ENG) evaluations and whose examinations yielded normal results. Prior to their examinations the subjects were instructed to discontinue all but essential medications and to abstain from alcohol consumption for 48 hours prior to the examination. As a part of their evaluation, all subjects received conventional pure tone and speech audiometry.

Electrodes (ICS EOG disposable or Beckman Ag/AgCl) were attached lateral to the outer canthus of each eye to record horizontal eye movements, and above and below one eye on a plane bisecting the pupil during primary gaze to record vertical eye movements. A ground electrode was placed on the forehead. The skin surface was prepared in a conventional manner and interelectrode impedances were always less than 5,000 Ω. Eye movements were recorded with a commercially available two-channel ENG (Tracoustics RN 260) that provided differential amplification, bandpass filtering (0.05 to 10 Hz), and a hard copy record (10 mm/s paper speed) of the nystagmus signal.

The ENG examination consisted of the following tests: 1) saccade system testing, 2) tests for horizontal and vertical gaze-evoked nystagmus, 3) optokinetic nystagmus with a series of light-emitting diodes (LEDs) that moved sequentially through the visual field at a rate of 15°/s and 30°/s, 4) pursuit system testing, 5) dynamic position testing (Dix-Hallpike maneuver) for all subjects who were free of disease involving the cervical vertebrae, 6) static position testing to detect position-induced nystagmus in the sitting, supine, right and left lateral, and head-hanging positions, and 7) caloric testing according to the Fitzgerald and Hallpike procedure[1] in a manner described by Barber and Stockwell,[21] or a monothermal warm water caloric test that has been described previously by Jacobson and Means.[22] Alertness was maintained by having the subjects perform counting or naming exercises throughout the caloric examination.

The caloric examination was conducted with the subject in the supine position with the head elevated 30°. The test was conducted in a semidarkened room with the subject's eyes closed. A commercially available caloric irrigator was used (Tracoustics RN 251a). Cool (30°C) and warm (44°C) water irrigations were carried out over a 40-second period for a total water volume of 250 mL. Alerting tasks were begun halfway through the irrigations and were continued throughout the examination. Fixation suppression ability was evaluated at the point of maximum nystagmus SPEV (determined visually by the clinician) by having the subjects open their eyes and fixate on an LED target that was located on the ceiling at primary gaze 2 m away. The visual inspection of the nystagmus record for the maximum velocity was facilitated by use of the second ENG recording channel (ie, vertical eye movement channel) to display the derived SPEV of the horizontal nystagmus. Nystagmus directional preponderance and unilateral weakness were calculated with the formulas developed by Jongkees.[23] Fixation suppression was calculated with a modification of the fixation index (FI) formula developed by Demanez and Ledoux[4]: FI = SPEV(EO)/SPEV(EC), where SPEV(EO) represents the average SPEV that occurred for 5 seconds after the eyes were opened and fixated (allowing up to 2 seconds for visual fixation to occur) and SPEV(EC) represents the average SPEV that occurred for 5 seconds before the eyes were opened at the point of greatest nystagmus velocity. The calculation of FS as described in this study is illustrated in Fig 1.

Any individual who did not abide by the pretest instructions or showed evidence of having vestibular or ocular motor system deficits during ocular motor, positional, or caloric testing was excluded from data analysis. Calculations of nystagmus velocity and FS were conducted by hand and by one examiner (K.G.H.). Each subject yielded between two and four ENG records. Four subjects received monothermal caloric examinations and 19 subjects received bithermal caloric examinations. Thus, 19 subjects yielded two warm and two cool caloric FIs and four subjects yielded only two warm caloric FIs. These data were subjected to separate analyses in an attempt to determine whether warm caloric stimulation resulted in larger nystagmus SPEVs and poorer FIs than did cool caloric stimulation.

RESULTS

The average maximum SPEVs following the cool and warm caloric irrigations were 20.31°/s and 29.59°/s, respectively (see Table). A *t* test for matched samples ($n = 38$) showed these differences to be statistically significant ($t = 5.83$, $df = 37$, $p < .001$). The mean FIs following cool and warm

RESULTS OF CALORIC IRRIGATIONS (N = 38)

	Maximum SPEV (Prefixation)		*Fixation Index*	
	44°C	*30°C*	*44°C*	*30°C*
$\bar{x}$	29.59	20.31	0.18	0.06
SD	12.23	7.60	0.18	0.12
+ 2.5 SD upper limit			0.62	0.35
t	5.83		4.08	
p<*	.001		.001	
df	37		37	

SPEV — slow-phase eye velocity.

*Two-tailed probability test.

caloric irrigations were 0.06 and 0.18, respectively (see Table). A *t* test for matched samples showed these differences to be statistically significant ($t = 4.08$, df = 37, $p < .001$). Typical results from one subject are given in Fig 2.

A Pearson product-moment correlation analysis was conducted to determine whether the temperature-dependent differences in FS were influenced by subject age. Separate analyses were conducted for cool (n = 38) and warm (n = 46) caloric stimulation. The results of these analyses showed that although the subject population was small a significant relationship existed between subject age and FS (r = .29, df = 44, p = .05) following warm caloric stimulation. A nonsignificant relationship was found between subject age and the magnitude of FS following cool caloric stimulation (r = .11, df = 36, p > .50).

DISCUSSION

The results of this investigation have shown that the caloric irrigating medium that produced the greatest stimulation of the vestibulo-ocular reflex (VOR) was associated with the poorest VOR cancellation. That the warm caloric stimulation produced a larger SPEV is not surprising. It is well known that warm air or water stimulation produces a larger maximum SPEV than cool air or water stimulation.[17,24] It was unexpected to find that the differences in FS ability were associated with relatively small differences in the mean maximum SPEV for the cool and warm caloric irrigations (20.31°/s and 29.59°/s, respectively). It may be possible to explain these differences if the assumption that FS is mediated by the pursuit pathway is correct. The upper limit for normal pursuit gain (ie, a gain of 1.0) is 30°/s for target excursions of ±10° from primary gaze.[18] A pursuit of 1.0 for elderly subjects is not seen for target velocities greater than 5°/s.[18] The mean age of the subjects in the study conducted by Sharpe and Sylvester[18] was 72 years. The mean age of the subjects who participated in the present investigation was 58 years. A substantial number of elderly subjects were included in the present subject pool (age range, 35 to 74 years). In fact, 61% of the subject population was 60 years of age or older. Thus, it is likely that the mean difference in the warm and cool caloric nystagmus maximum SPEV of 9°/s was sufficient to cause the temperature-dependent, statistically significant differences in FS.

There is considerable disagreement about the neuronal mechanisms that subserve FS. It has been reported previously that there is a close relationship between the upper limits of smooth pursuit velocity in a given individual and the upper limit of FS during rotatory stimulation.[12,13,25] Additionally, it has been noted by other investigators that patients who have CNS diseases affecting the cerebellar flocculus, pons, and mesencephalic visual centers have impaired smooth pursuit and FS abilities.[6,10,26,27] Deficits in smooth pursuit and FS have been dem-

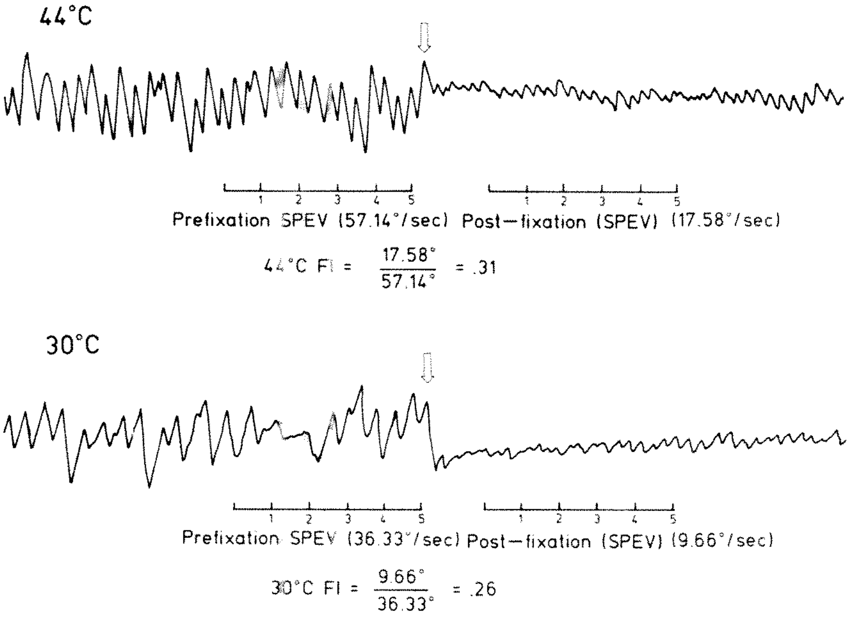

Fig 2. Electronystagmograms obtained from one subject illustrating trends observed in present investigation. Warm irrigation elicited larger prefixation slow-phase eye velocity (SPEV) than cool irrigation, and was followed by poorer fixation index (FI).

onstrated in flocculectomized rabbits and monkeys.[8,28] These observations have formed the support for the argument that FS is wholly or substantially mediated by the pursuit system.

However, there is equally convincing evidence that pursuit and FS are mediated by functionally distinctive pathways. For example, it is possible for animals who have poorly developed pursuit systems (ie, the cat) to suppress the VOR at head velocities of up to 60°/s.[14] Additionally, human subjects who normally cannot generate pursuit movements in darkness (ie, in the absence of a target) can suppress the VOR during rotation by "imagining" a visual stabilization point directly in front of them.[29] It is believed currently that if the pursuit system does not mediate FS completely then at least the pursuit and FS systems share a common circuitry.[14]

Other factors may have contributed to the subjects' poorer FS performance for the warm stimuli. The greater SPEV for warm calorics may have been associated with a stronger perception of dizziness. Accordingly, the subjects may have been less inclined to perform the FS task to the best of their abilities following the warm caloric stimulation.

Based on the present data it is suggested that separate upper limits for FS should be established for cool and warm caloric stimulation. It is also suggested that a more detailed investigation of the effects of age on FS needs to be conducted. This investigation would require a large age-stratified subject sample.

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EFFECTS OF RECURRENT LARYNGEAL NERVE TRANSECTION AND VAGOTOMY ON RESPIRATORY CONTRACTION OF THE CRICOTHYROID MUSCLE

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The cricothyroid muscle (CT) appears to be an accessory muscle of respiration. Phasic inspiratory contraction is stimulated by increasing respiratory demand. Reflex activation of the CT may be responsible for the paramedian position of the vocal folds, and hence airway obstruction, in patients with bilateral recurrent laryngeal nerve (RLN) paralysis. Previous research has demonstrated the influence of superior laryngeal nerve (SLN) afferents on CT activity. The present study addresses the effects of vagal and RLN afferents. Electromyographic activity of the CT and right posterior cricoarytenoid muscle was monitored in anesthetized cats during tracheotomy breathing and in response to tracheal or upper airway occlusion in the intact animal. This was repeated following left RLN transection, bilateral vagotomy, and bilateral SLN transection. Vagotomy abolished CT response to tracheal occlusion and markedly reduced the response to upper airway occlusion. Vocal fold position following RLN transection appeared to correlate with CT activity; however, observed changes were minor.

KEY WORDS — larynx, recurrent laryngeal nerve paralysis, reflex.

INTRODUCTION

The role of the cricothyroid muscle (CT) in respiration is not well understood. The CT appears to be an accessory muscle of respiration, subject to complex control.[1] Some authors have reported phasic CT contraction during inspiration,[2-4] while others have observed expiratory activity[5-7] or biphasic contraction.[8-11] The variations in observations of CT activity can be attributed largely to differences in experimental conditions. Cricothyroid muscle activity is affected profoundly by respiratory variables[8,9] as well as level of consciousness.[10,11]

The control of CT activity is clinically important in the pathophysiology of recurrent laryngeal nerve (RLN) paralysis. In this condition, all intrinsic laryngeal muscles are paralyzed except for the CT, which is innervated by the superior laryngeal nerve (SLN). It is believed that continued contraction of the CT maintains the vocal fold near the midline rather than in the more lateral, cadaveric position of complete laryngeal paralysis.[12] The precise mechanism of vocal fold adduction by the CT has not been addressed previously, to our knowledge. Possible mechanisms include resting muscle tone, normal phasic contraction with respiration, or reflex contraction in response to respiratory or proprioceptive cues. If respiratory reflex CT contraction plays an important role, then it is conceivable that modification of the afferent limb of the reflex could induce changes in vocal fold position, and hence the symptoms, in patients with RLN paralysis.

Animal studies have shown that inspiratory CT contraction is stimulated strongly by negative upper airway pressure and that this effect is mediated by sensory receptors in the internal branch of the SLN. There is evidence that other stimuli affect respiratory activity of the CT. Both stimulation of laryngeal flow receptors and tracheal occlusion (TO) have been associated with increased CT activity.[1] Clinical experience suggests that vagal afferents also may be important in modulating CT function. Intrathoracic RLN injury can result in an intermediate position of the vocal fold, implying inactivity of the CT. It has been hypothesized that concomitant injury to the vagus nerve is responsible for this effect.[13]

The present study was designed to determine the effects of left RLN transection and bilateral vagotomy on CT and right posterior cricoarytenoid muscle (PCA) respiratory activity. Cricothyroid muscle electromyographic (EMG) activity also was correlated with vocal fold position to determine the mechanism of vocal fold adduction following RLN paralysis. It was hypothesized that 1) vagotomy would diminish the response of the CT to airway occlusion, 2) a paramedian vocal fold position following RLN transection would correlate with a high level of CT EMG activity, and 3) an intermediate vocal fold position would be associated with a low level of CT activity.

METHODS

Preparation. Ten spontaneously breathing anesthetized cats were used as subjects in this study. Cats initially were subdued with intramuscular ketamine (25 mg/kg), then anesthetic (chloralose,

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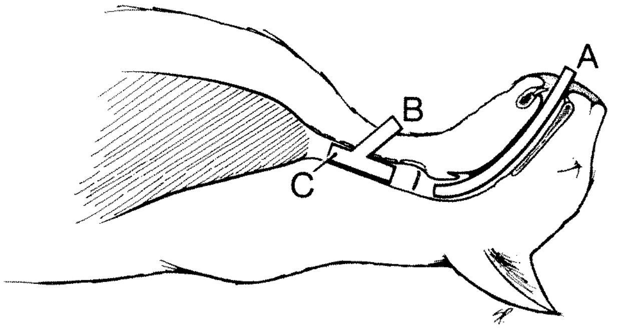

Fig 1. Experimental setup. Upper airway is stented open by rigid polyethylene tube sutured to epiglottis. Epoxy mask creates airtight seal of nose and mouth around tube. Breathing can be directed through side arm of tracheal cannula (B) or upper airway stent (A). Upper airway occlusion is produced by simultaneously closing points A and B. Tracheal occlusion is produced by inflating balloon at point C.

0.1 g/kg, and urethane, 1.0 g/kg) was administered intravenously. Additional anesthetic was administered as needed through the intravenous line to maintain a stable plane of surgical anesthesia. Core body temperature was monitored with a rectal thermometer and maintained within normal limits using a heat lamp and a blanket as needed. Cats were euthanized at the end of the study, without regaining consciousness.

The larynx and trachea were exposed using a vertical midline incision in the neck. Both RLNs were identified, preserved, and tagged with silk sutures loosely passed around the nerves. The left cervical vagus nerve, the right vagus nerve below the takeoff of the RLN,[14] and the sensory branch of both SLNs were identified and tagged in the same fashion.

A cannula with a side arm was placed into the trachea and secured in position with ligatures around the trachea to ensure a tight seal. The upper airway was stented open above the larynx using a large-bore plastic tube. This tube was sutured to the epiglottis to maintain patency of the supraglottis. A No. 3 F Mylar solid-state pressure transducer was introduced through a nostril of the cat and was advanced into the midthoracic esophagus to monitor intrathoracic pressure. The mouth and nose then were sealed with an epoxy mask around the airway stent and transducer as described by Sant'Ambrogio et al[15] (Fig 1).

Hooked-wire electrodes were placed into the right PCA and the left CT. Electrical activity from the CT and PCA was filtered, rectified, integrated over 50-ms intervals, and displayed on a Gould-Brush recorder, along with intrathoracic pressure.

A 0° Karl Storz arthroscope and xenon light source were used to observe the larynx. Photographs were taken with an Olympus OM-2N camera.

Protocol. The animals were observed during spontaneous breathing through the tracheotomy tube (TB), during single-breath TO, and during simultaneous occlusion of the upper airway tube and the side arm of the tracheal cannula to create single-breath upper airway occlusion (UAO). Inspiratory effort (as evidenced by intrathoracic pressure measurement) was compared for the two types of occlusion to assure that the upper airway seal was complete.

Three trials of each type of occlusion were performed, allowing sufficient time between occlusions for the breathing pattern and EMG activity to return to baseline. Progressive neural lesions then were created in this order: 1) transection of the left RLN, 2) transection of the left cervical vagus nerve and the right vagus nerve below the takeoff of the right RLN, and 3) transection of the internal branches of both SLNs. Occlusion trials were repeated after each neural lesion was made.

Data Analysis. Peak inspiratory EMG activity was measured in arbitrary units above electrical silence. Activity during each occlusion was expressed as a percentage of the peak inspiratory activity seen during breathing through the TB.

The timings of photographs of the larynx were indicated by an event marker on the strip chart recorder. Photographs were classified as inspiratory or expiratory and further categorized according to the level of CT activity at the time the photographs were taken. Statistical significance of differences in EMG activity was established by analysis of variance.

RESULTS

In two of the cats, a total seal by the epoxy mask could not be achieved and there was an air leak resulting in incomplete UAO. Data analysis is limited to those cats in which there was no leak and esophageal pressure with inspiration was equal during TO and UAO.

Under stable anesthesia with spontaneous breathing through the TB, prior to any neural lesions, both the CT and PCA showed phasic contraction with inspiration (Fig 2A). Telescopic examination of the larynx confirmed vocal fold abduction during inspiration and gradual adduction with expiration. Single-breath occlusion of the airway was associated with increased EMG activity of both muscles in inspiration. Upper airway occlusion had a much greater effect than TO on CT contraction. Peak inspiratory activity of the CT during UAO increased to an average of 824% of the level seen during tracheal breathing (Fig 3). In contrast, TO produced an increase in CT activity of only 292%. The PCA was less sensitive than the CT to airway occlusion. In the intact animal, TO produced an increase in inspiratory PCA activity to 177% of that during TB, while UAO resulted in a level of 212%.

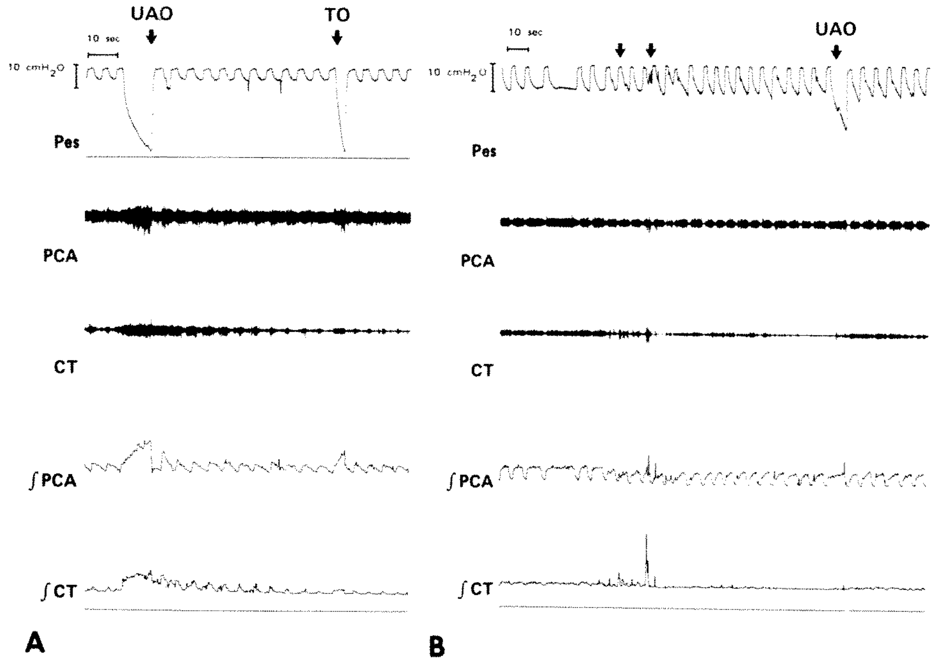

Fig 2. Laryngeal muscle activity. UAO — upper airway occlusion, TO — tracheal occlusion, Pes — esophageal pressure, CT — raw EMG activity in cricothyroid muscle, PCA — raw EMG activity in posterior cricoarytenoid muscle, ∫CT — rectified and integrated EMG activity in CT, ∫PCA — rectified and integrated EMG activity in PCA. A) UAO and TO generate equivalent inspiratory effort (esophageal pressure, Pes), but UAO is much more potent stimulus than TO for CT activity. B) Inhibition of CT activity by transection of left recurrent laryngeal nerve. First arrow — beginning of dissection in area, second arrow — recurrent nerve transection. CT activity is markedly reduced, but some activity can still be recruited by UAO (third arrow). Baseline activity in right PCA is unchanged.

The effect of progressive neural lesions on CT and PCA response to airway occlusion is depicted in Fig 3. Transection of the left RLN resulted in a temporary large decrease in CT activity (Fig 2B). After 30 minutes to 1 hour, CT activity gradually increased and stabilized at or near previous levels. Occlusion trials were performed on this state. Cricothyroid responses to both UAO and TO were diminished to 567% and 194%, respectively ($p<.001$). Right PCA response decreased to 165% for TO and 186% for UAO ($p<.001$).

Immediately following transection of both vagus nerves there was an apneustic breathing pattern with extremely negative intrathoracic pressure during inspiration and frequent inspiratory arrest of breathing effort. Tracheal obstruction no longer affected CT and PCA activity. Upper airway obstruction, however, produced some increase over baseline activity.

After 30 minutes to an hour, breathing became more regular and intrathoracic pressure changes returned to a normal range (Fig 4A). Data in Fig 3 are based on occlusion trials performed on this state. Neither the PCA nor the CT responded to TO, and responses of both muscles to UAO were significantly less than in the intact state or following left RLN transection ($p<.001$).

Following bilateral transection of the internal branch of both SLNs, there was no detectable CT or PCA response to single-breath occlusion of the airway at either level. Occlusion for multiple breaths led to increasing CT and PCA activity (Fig 4B).

A telescopic observation following RLN paralysis revealed that vocal fold position could be correlated with CT activity; the changes in position, however, were very small and difficult to quantify (Fig 5).

DISCUSSION

The results of this study demonstrate that in addition to SLN afferents, nerve fibers in the left RLN and both vagus nerves contribute to modulation of CT activity with respiration. The study also confirms the findings of other authors regarding the influence of SLN and vagal afferents on PCA activity.[16,17] Inspiratory activity of both the CT and PCA in response to UAO was diminished by RLN transection. Vagotomy abolished the response of both muscles to single-breath TO and significantly decreased the response to UAO. Sensory SLN transection abolished all remaining response to single-breath UAO. Prolonged occlusion still led to increasing CT and PCA activity, indicating that other

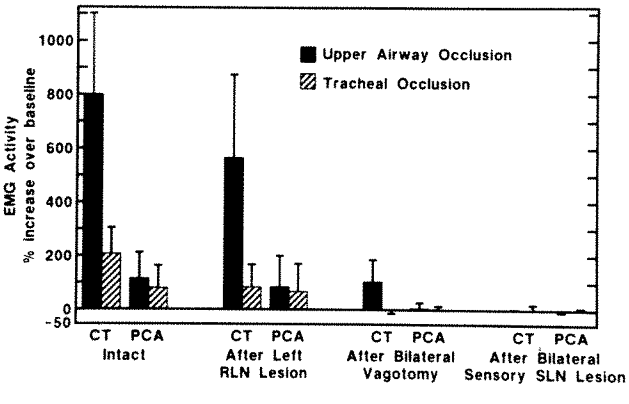

Fig 3. Effect of progressive neural lesions on laryngeal muscle response to airway occlusion. Peak inspiratory EMG activity of each muscle during upper airway occlusion or tracheal occlusion is expressed as percentage of activity in that muscle during quiet inspiration via tracheotomy (baseline activity). CT — cricothyroid muscle, PCA — posterior cricothyroid muscle, RLN — recurrent laryngeal nerve, Vagotomy — transection of left cervical vagus nerve, and right vagus nerve below takeoff of right RLN, SLN — sensory branch of superior laryngeal nerve.

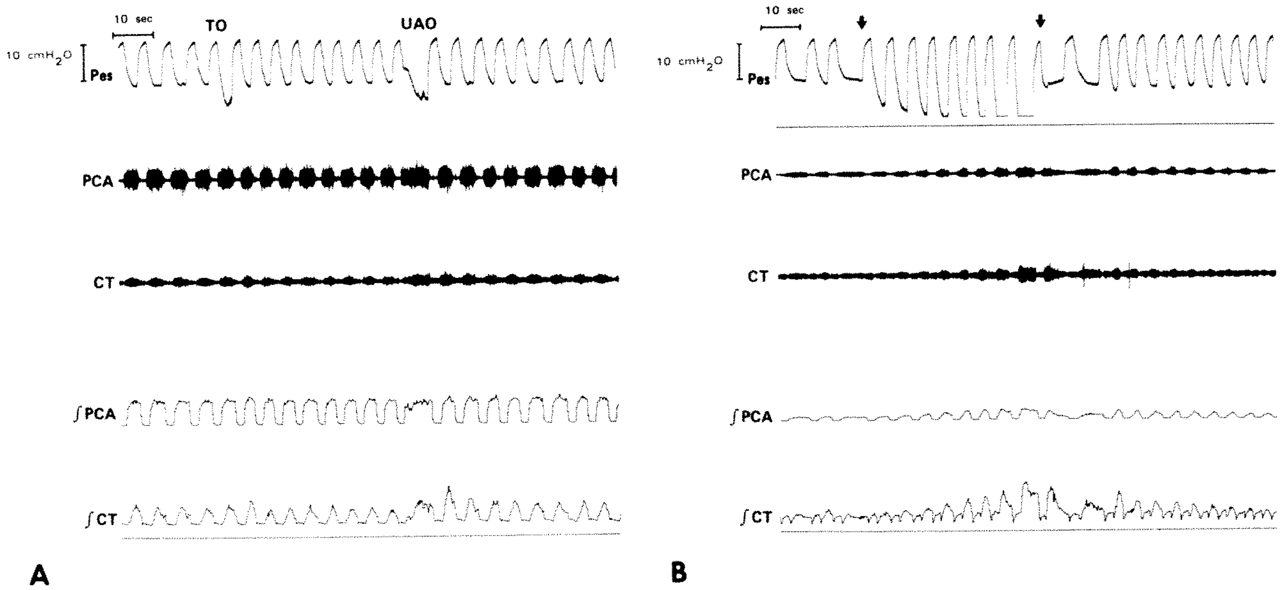

Fig 4. Effect of airway occlusion. (Symbols as in Fig 2.) A) Following bilateral vagotomy. TO has no effect on muscle activity during occluded breath. UAO still recruits CT activity. Also note increase in activity during breaths following occlusion. B) Following bilateral vagotomy and transection of sensory branches of both superior laryngeal nerves. Gain of EMG activity has been reduced. Note that first occluded breath does not affect muscle activity, but prolonged occlusion does. This may be due to changes in blood gas values.

pathways, presumably including chemoreceptors, also affect CT and PCA function.

It is unlikely that hypoxia or hypercapnia were responsible for the stimulatory effects of the brief airway occlusions. Although blood gases were not monitored during this study, pilot experiments indicated that the concentration of carbon dioxide in expired air does not increase significantly during one occluded breathing effort. Hypercapnia induced by administration of CO_2 in air does not appreciably increase CT or PCA activity until levels of expired CO_2 are over 5%. Finally, the effects of chemoreceptor stimulation can be estimated by the effects of airway occlusion following transection of the SLNs and vagus nerves (Fig 4B). Cricothyroid muscle and PCA activity was not increased until the third or fourth breathing effort.

A previous study in dogs indicated that SLN receptors appeared to have a very large effect on CT respiratory activity.[1] Following transection of both sensory SLNs, response to UAO decreased dramatically, and there was no observable difference in CT responses to UAO and TO. Thus, the large difference in CT responses to UAO and TO was attributed solely to the influence of the SLN.

Study of the effects of vagotomy on laryngeal muscle activity in dogs is not practical, because the RLN fibers exit the nerve intrathoracically. Hence, an RLN-sparing vagotomy can be accomplished only by thoracotomy, which introduces numerous other confounding factors. In cats, however, RLN fibers exit the right vagus nerve before entering the thorax. Thus, vagotomy may be performed transcervically without paralyzing the right hemilarynx. Cervical vagotomy on the left still requires RLN transection. In this experiment, left RLN transection was performed before vagotomy, not only to study the effects of the RLN lesion, but also to separate these effects from those of vagotomy.

In the present study, vagotomy had a greater effect than expected on the response of the CT to UAO. The persisting response to UAO before sensory SLN transection was much smaller than the difference between responses to UAO and TO in the intact animal. This suggests that vagal and SLN stimuli have a synergistic as opposed to merely additive effect on CT function, such that the response of the CT to negative upper airway pressure is influenced by vagal feedback.

Other studies have suggested that CT contraction in respiration may be enhanced directly by RLN transection.[14] The results of the study do not confirm this finding. Electromyographic activity during quiet tracheal breathing was diminished markedly immediately following transection of the left RLN, but gradually increased and stabilized over 30 minutes to an hour. After this recovery period, inspiratory CT activity during quiet tracheal breathing was not significantly different from that in the intact animal, but the response to UAO was diminished significantly. In a previous experiment in dogs, we reported the effect of cold blockade of the RLN on CT activity.[1] Inspiratory activity of the CT during use of a TB was not noticeably changed, but the response to UAO was diminished somewhat. The profound temporary inhibition of CT activity seen with RLN transection was not seen with cold blockade. It is possible that cutting the RLN

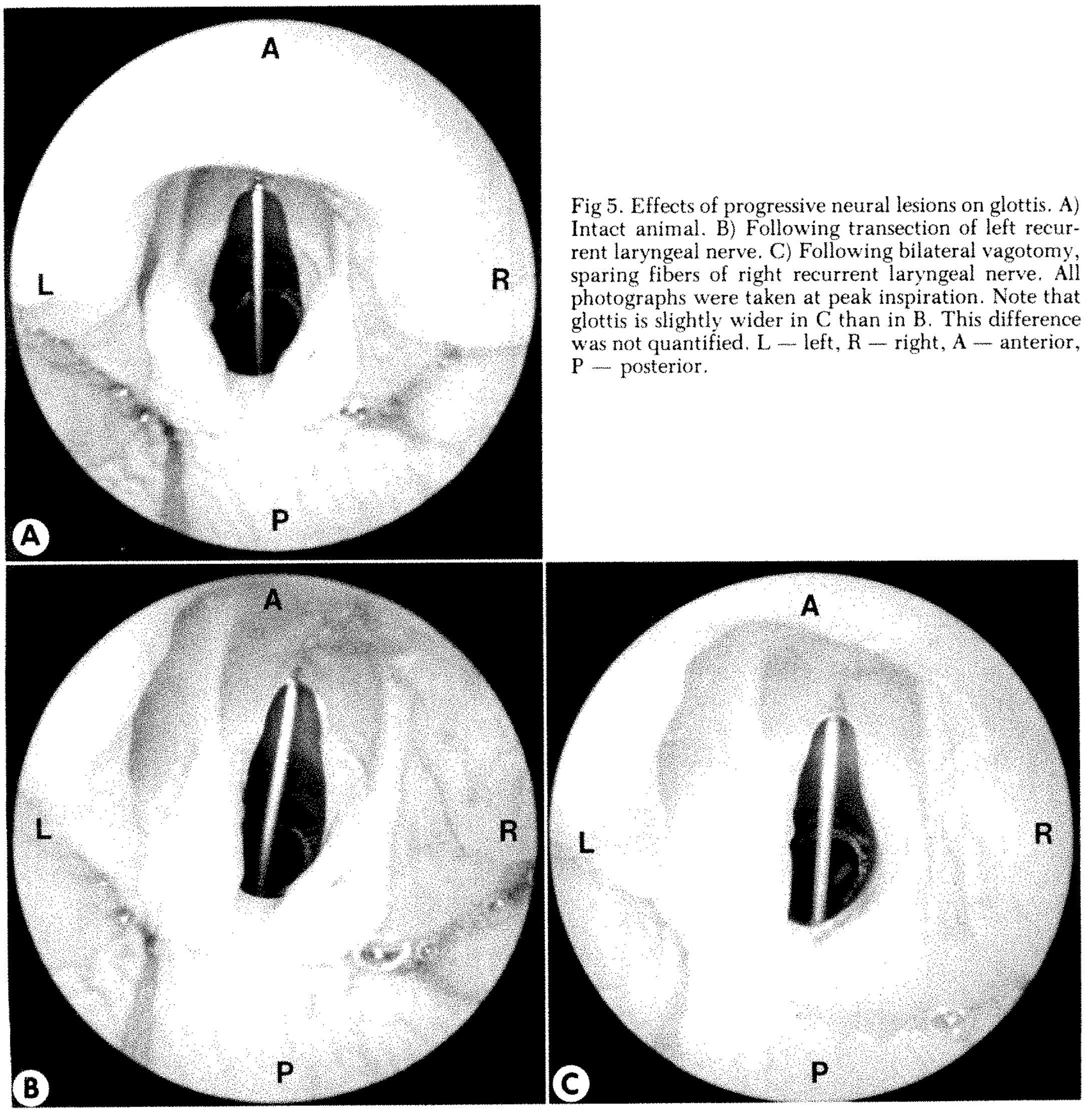

Fig 5. Effects of progressive neural lesions on glottis. A) Intact animal. B) Following transection of left recurrent laryngeal nerve. C) Following bilateral vagotomy, sparing fibers of right recurrent laryngeal nerve. All photographs were taken at peak inspiration. Note that glottis is slightly wider in C than in B. This difference was not quantified. L — left, R — right, A — anterior, P — posterior.

stimulates some fibers, leading to central inhibition. The same excitatory effect would not be expected with cold blockade.

The difference between our observations of the effect of RLN transection and the results of previous studies possibly could be explained by differing experimental conditions. In our studies, the animals breathed through a tracheotomy or a stented upper airway, which resulted in minimal upper airway resistance. In these conditions, transection or cold blockade of the RLN produced no significant change in upper airway pressures. In the studies of Fukuda and Kirchner,[14] animals breathed through an unstented upper airway and therefore baseline upper airway resistance was greater.[14] Under these conditions, paralysis of the RLN could have increased airway resistance further, and this may have been sufficient to stimulate CT activity.

The decreased response to UAO following left RLN transection suggests that there are afferent fibers in the RLN that stimulate CT contraction. These fibers could modulate subglottic or tracheal pressure sensation or perhaps laryngeal proprioception.

Vagotomy significantly influenced both PCA and CT activity. The vagus nerve carries fibers from intrapulmonary stretch receptors that respond to lung inflation. In the classical Hering-Breuer reflex, lung inflation inhibits inspiratory activity of the diaphragm. Under normal breathing conditions, this reflex may not be significant. The effect of vagal afferents on upper airway muscles appears to be somewhat different. Upper airway muscles normally are inhibited during inspiration by graded, vagally mediated sensation of lung inflation.[18] Airway occlusion prevents lung inflation and vagotomy blocks its sensation; both should lead to a marked increase in inspiratory activity. Airway occlusion blocks phasic stimulation of stretch receptors. Vagotomy should have a more profound effect since it abolishes not only phasic but also tonic stimuli. In the present study, acute vagotomy produced the expected increase in CT and PCA inspiratory activity far above that seen during airway occlusion. After a period of adaptation, however, breathing resumed normal levels, presumably because of central adaption to the lack of vagal input. The response of PCA and CT to UAO at this time was markedly depressed, and the response to TO was abolished.

These results seem to conflict with previous reports of the effect of vagotomy on CT activity. Fukuda and Kirchner[14] reported that in the presence of RLN paralysis during breathing through the upper airway, compression or topical anesthesia of both vagus nerves abolished the phasic contraction of the CT. They concluded that vagal afferents stimulated CT activity. A later study by Sasaki et al[19] showed no effect of vagotomy on CT activity; however, in the latter study, only the right vagus nerve was transected; thus fibers in the left vagus nerve were still intact.

Position of the vocal folds in this study appeared to correlate with EMG activity of the CT. The observed differences in vocal fold position with changes in CT activity were extremely small, and it is doubtful that they produced any significant changes in laryngeal resistance. This observation, however, was based on qualitative assessment of endoscopic observations and photographs. This position of the telescope with reference to the glottis was not standardized; hence the change in glottic dimension could not be assessed quantitatively. Further studies measuring glottal resistance or use of continuous video recording from a fixed reference position could determine whether or not changes in reflex CT contraction have a significant effect on vocal fold position and laryngeal resistance immediately following RLN paralysis.

These results appear to conflict with the clinical observation that in patients with laryngeal paralysis, vocal fold position is paramedian when the CT is intact (RLN injury) and significantly more lateral when the CT also is paralyzed (RLN plus SLN). The present study, however, involved only acute effects. It is possible that the effect of the CT on position does not occur immediately, but develops with time. Further studies in animals with chronic paralysis would be required to explore the possibility of late changes.

CONCLUSIONS

Cricothyroid muscle contraction in phase with respiration is modulated by afferent fibers in the vagus nerve as well as the SLN and RLN. Stimuli from the vagus nerve and SLN appear to be synergistic. Other pathways, such as chemoreceptors, also are implicated. The results of these studies are consistent with the hypothesis that active CT contraction, rather than resting muscle tone, affects vocal fold position following RLN paralysis. The observed changes in position, however, were extremely small. The functional significance of CT reflexes remains to be determined.

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EFFECTS OF NITROGEN MUSTARD-N-OXIDE ON IONIC ACTIVITIES OF INNER EAR FLUID AND IONIC PERMEABILITIES OF THE COCHLEAR PARTITION IN THE GUINEA PIG

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The effect of nitrogen mustard-N-oxide (NMO) on the endocochlear potential (EP) was investigated from the aspect of the ion concentrations and permeabilities in the cochlea. Compared with the untreated animals, in NMO-treated animals 20 to 30 hours after administration, the EP was decreased (30.8 ± 3.5 mV in NMO versus 82.4 ± 1.6 mV in control), the K^+ concentration in perilymph of the scala tympani was increased (8.2 ± 1.0 m*M* versus 5.3 ± 0.7 m*M*), the K^+ concentration in endolymph was decreased (128.5 ± 10.6 m*M* versus 157.9 ± 7.9 m*M*), and the Na^+ concentration in endolymph was increased (9.6 ± 3.6 m*M* versus 2.5 ± 0.4 m*M*). The permeability coefficient for Na^+ of the cochlear partition in the NMO-treated animals significantly decreased, while that for Cl^- significantly increased. The negative EP, which presumably exists in the normal state, diminished further (−2.7 mV versus −27.8 mV), and the calculated electrogenic potential of the EP was depressed remarkably (33.5 mV versus 110.2 mV). The results suggest that the effects of NMO involved changes in ion permeabilities of the partition and the inhibition of electrogenic transport processes in the cochlea.

KEY WORDS — anoxia, electrogenic potential, endocochlear potential.

INTRODUCTION

Light and electron microscopic observations[1-3] showed that nitrogen mustard-N-oxide (NMO) induced selective damage of the outer hair cells while the other sites, including the stria vascularis, were scarcely affected. On the other hand, the endocochlear potential (EP), which is widely accepted as being generated by the stria vascularis, showed a significant decrease following the administration of NMO.[3,4] Although Asakuma et al[3] demonstrated that the EP initially reached a minimum level within a few days and then gradually recovered to the normal level within a few weeks in NMO-treated animals, they could not find a cause of the EP depression by measuring the electrical resistance of the cochlear partition or by an electron microscopic study of the stria vascularis. So far, the cause of the EP depression induced by NMO is still unknown. Furthermore, it is highly possible that the effect of anoxia on the EP is different in normal and NMO-treated animals, since in kanamycin-treated animals, the EP under anoxic conditions ranged negative values (whose magnitude was smaller than those found in untreated animals) to positive values.[5,6]

In the present experiment, we aimed at elucidating the mechanism of EP depression by measuring the ion (K^+, Na^+, Cl^-) activities of inner ear fluid and the ion permeabilities of the cochlear partition in NMO-treated cochleas under normally ventilated and anoxic conditions.

MATERIALS AND METHODS

Albino guinea pigs with a normal Preyer's reflex weighing between 300 and 400 g were used. Fifteen guinea pigs were anesthetized by respired diethyl ether and were given 60 mg/kg of NMO via a right jugular vein. These animals were subjected to the electrophysiologic measurements after 20 to 30 hours. Sixteen animals were used as controls.

The left tympanic bulla was exposed with a ventrolateral approach. A double-barreled ion-selective microelectrode at first was inserted into the scala tympani of the basal turn through the round window, and the ionic activity in perilymph was measured. Then, the microelectrode tip was advanced further into the scala media and was held in position. Permanent anoxia was induced by interrupting artificial ventilation, and the change in ion activity in the endolymph was monitored. Then the change in ion activity in the perilymph induced by anoxia was observed by pulling out the microelectrode tip to the perilymph after 30 minutes.

The calibration method and the recording system of the microelectrodes have been described elsewhere.[7,8] Ion exchangers and internal solutions filled in the ion-selective barrel were Corning 477317 (Corning Medical) and 0.5 *M* KCl for the K^+ microelectrode, ETH 227 (Science Trading) and 0.5 *M* NaCl for the Na^+ microelectrode, Corning 477913 (Corning Medical) and 0.5 *M* KCl for the

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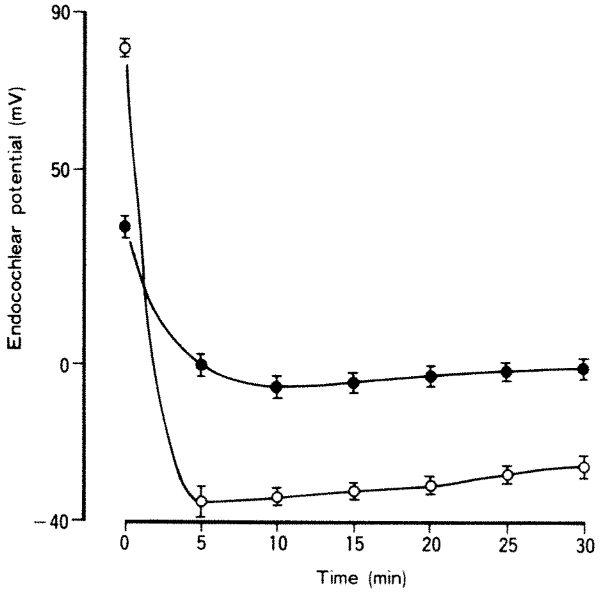

Fig 1. Time course of endocochlear potential (mean ± standard error of mean) during permanent anoxia in normal (open circle) and nitrogen mustard-N-oxide–treated (closed circle) animals.

Cl^- microelectrode, and ETH 1001 (Science Trading) and 0.1 M $CaCl_2$ for the Ca^{2+} microelectrode. The calibration of the electrodes was performed according to methods previously reported.[7-9] The selectivity coefficients, except for the Ca^{2+} microelectrode, were obtained at an ion concentration of 150 mM. The selectivity coefficient in the Ca^{2+} microelectrode was measured at 10^{-5} M Ca^{2+} by use of the separate-solution method.[10] When the measurement in the cochlear fluids was finished, a calibration in vivo was performed by immersing the electrode into a standard solution of known composition in the tympanic bulla. During the experiments, the rectal temperature of the animal was kept at 37.5°C ± 0.5°C.

Permeability of ion i (P_i) of the endolymph-perilymph barrier was calculated according to the equation described by Konishi and Salt.[11]

$$P_i = RTG_i/z^2F^2\bar{c} \qquad (1)$$

where z is valence, F is Faraday's constant (96,500 joules/V/equivalent), R is gas constant (8,314 joules/mole/degrees absolute temperature), and T is degrees absolute temperature. P_i estimated from this equation (equation 1) does not necessarily indicate true membrane permeability, but is rather a parameter that is related to the composite permeabilities of the membranes lining the cochlear duct and tight junctions.

The symbol $\bar{c}$ represents the mean ion concentration in the membrane defined by the following equation.

$$\bar{c} = (C_e - C_p)/\ln(C_e/C_p)$$

G_i (the ion conductance) is defined by the following equation (equation 2).

$$G_i = zFJ_i/[RT/zF \cdot \ln(C_e/C_p) + \Delta\psi] \qquad (2)$$

where C_p and C_e represent the ion concentrations in perilymph and endolymph, respectively. The time course of each ion concentration in perilymph under the anoxic condition was assumed to exhibit a linear change during the 0- to 30-minute period,[11-13] so that the concentration for a given time was interpolated from the initial and 30-minute values. $\Delta\psi$ is the potential difference between the endolymph and perilymph, and J_i is the net ion flux into or out of the endolymph defined by

$$J_i = V_e \cdot dc_e/dt$$

where Ve represents the volume of endolymph that was estimated to be 2 mm^3.

The results were recorded as mean ± standard error of the mean. Statistical analysis was performed using Student's t test, and p values less than .05 were considered to be statistically significant.

RESULTS

Properties of Double-Barreled Ion-Selective Microelectrodes. The K^+ microelectrodes (n = 11)

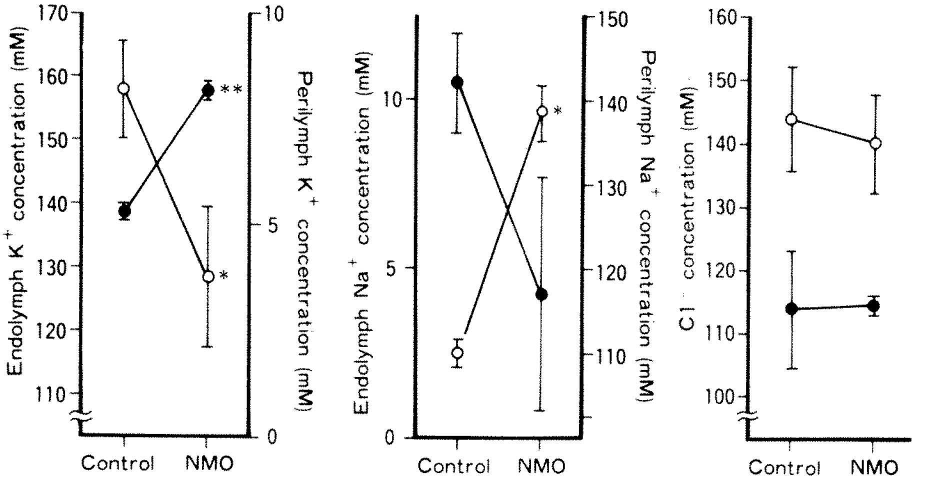

Fig 2. Ionic concentrations (mean ± standard error of mean) of endolymph (open circle) and perilymph (closed circle) in normal and nitrogen mustard-N-oxide–treated animals. * —p < .01, ** —p < .05.

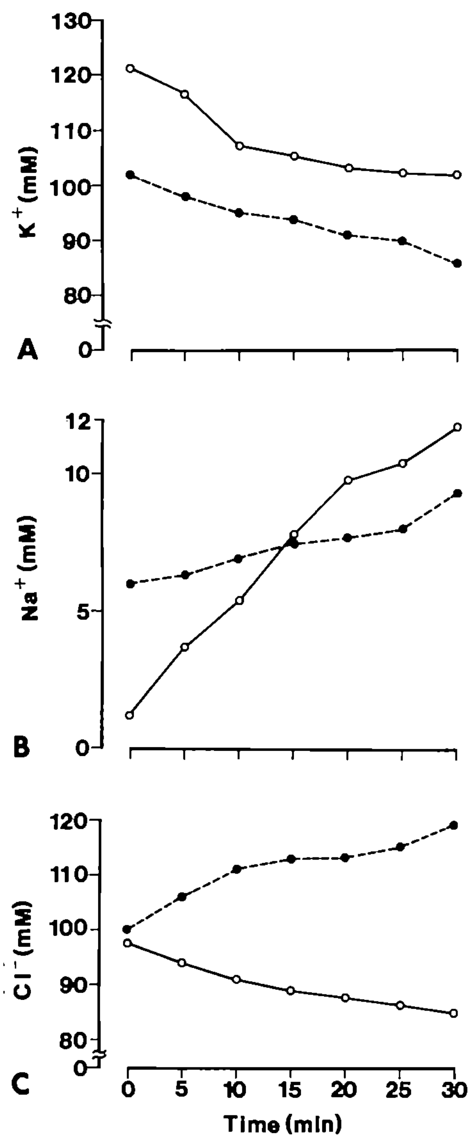

Fig 3. Representative changes of endolymph ion activities during permanent anoxia in normal (open circle) and nitrogen mustard-N-oxide–treated (closed circle) animals. A) K$^+$, B) Na$^+$, C) Cl$^-$.

had an average slope of 53.8±1.0 mV/tenfold change and the selectivity coefficient over Na$^+$ of 0.010±0.001. The Na$^+$ microelectrodes (n = 8) had an average slope of 52.7±1.0 mV/tenfold change and the selectivity coefficients over K$^+$ of 0.013±0.002 and over Ca^{2+} of less than 2.0. The Cl$^-$ microelectrodes (n = 9) had an average slope of 55.6±1.7 mV/tenfold change and the selectivity coefficient over HCO$_3^-$ of 0.12±0.01. The Ca^{2+} microelectrodes (n = 3) had a slope of 26.3±0.3 mV, and the interference by other cations was negligible, similar to that previously reported.[8]

Endocochlear Potential and Effect of Anoxia. The EP (30.8±3.5 mV, n = 12) in NMO-treated animals showed a significant depression (p<.001) compared with that in controls (82.4±1.6 mV, n = 16). The time course of the effect of anoxia on the EP is shown in Fig 1, where the average EP values are plotted every 5 minutes. In control animals (n = 11), the EP rapidly declined and reached the minimum value of −35.8±4.5 mV at 5 minutes. Thereafter the potential gradually returned toward zero. On the other hand, the EP in NMO-treated animals (n = 10) showed a relatively slow decrease by anoxia and reached the minimum at 10 minutes: -5.7±3.0 mV, which was significantly higher than that in controls (p<.001). After 10 minutes the EP showed an increase, with a more sluggish pattern than that in controls.

TABLE 1. MEAN PERMEABILITY COEFFICIENTS OF COCHLEAR PARTITION DURING PERMANENT ANOXIA

Period (min)	*In Control Animals*			*In NMO-Treated Animals*		
	K$^+$	*Na$^+$*	*Cl$^-$*	*K$^+$*	*Na$^+$*	*Cl$^-$*
10-15	8.221	0.526	1.377	2.317	0.125	7.233
15-20	4.442	0.357	1.376	4.035	0.213	10.225
20-25	5.634	0.272	1.687	2.049	0.116	5.900
25-30	5.510	0.439	2.047	2.599	0.104	7.243

Permeability coefficients are reported x10^{-4} cm^3/min.
NMO — nitrogen mustard-N-oxide.

Ionic Concentrations in Perilymph and Endolymph. The K$^+$, Na$^+$, and Cl$^-$ concentrations in perilymph and endolymph are shown in Fig 2. In comparison with control animals, NMO-treated animals showed a significant decrease of K$^+$ concentration in endolymph (p<.01), an increase of K$^+$ concentration in perilymph (p<.05), and an increase of Na$^+$ concentration in endolymph (p<.01). The Na$^+$ concentration in perilymph had a tendency to decrease, but it was not significant. There was no difference in Cl$^-$ concentrations between control and NMO-treated animals. The endolymph Ca^{2+} concentration in NMO-treated animals was 4.9±7 x 10^{-4} *M* (n = 3), which created an error signal in the Na$^+$ microelectrode corresponding to 1.0 m*M* Na$^+$. The values of Na$^+$ concentration shown in Fig 2 included this correction. However, the errors by interference of Ca^{2+} on the Na$^+$ activity measurements in perilymph of two animal groups and in endolymph of the control group was less than 5% on the basis of our previous report.[8] In both groups, the Cl$^-$ concentrations in endolymph were significantly higher than those in perilymph (p<.02).

Effect of Anoxia on Ion Activities. Figure 3 shows the time course of the changes in the ion activities in endolymph induced by anoxia. In NMO-treated an-

TABLE 2. PERMEABILITY COEFFICIENTS 25 to 30 MINUTES AFTER ANOXIA

	In Control Animals	*In NMO-Treated Animals*	*Student's t Test*
K$^+$	5.510±1.262 (4)	2.599±0.527 (4)	NS
Na$^+$	0.438±0.075 (4)	0.104±0.020 (3)	p<.01
Cl$^-$	2.047±0.101 (3)	7.244±0.891 (3)	p<.02

Permeability coefficients are mean±standard error of mean, x10^{-4} cm^3/min. Numbers in parentheses are numbers of animals.
NMO — nitrogen mustard-N-oxide, NS — not significant.

TABLE 3. ION CONDUCTANCES 25 TO 30 MINUTES AFTER ANOXIA

	In Control Animals	*In NMO-Treated Animals*	*Student's t Test*
K^+	0.276±0.121 (4)	0.130±0.071 (4)	NS
Na^+	0.021±0.007 (4)	0.004±0.002 (3)	NS
Cl^-	0.173±0.014 (3)	0.519±0.142 (3)	$p<.02$

Conductances are mean±standard error of mean, siemens. Numbers in parentheses are numbers of animals.

NMO — nitrogen mustard-N-oxide, NS — not significant.

imals, the rate of increase in Na^+ was clearly slower than that in controls. The Cl^- activity changed in an opposite direction under the anoxic condition, ie, a decrease in controls and an increase in NMO-treated animals. The Ca^{2+} activity in endolymph was increased slightly by anoxia, so that the influence on the Na^+ activity measurements was judged to be negligible. Mean permeability coefficients calculated by equation 1 for these three ions are shown in Table 1, where the permeability coefficients during the 0- to 10-minute period are excluded because the electrogenic component of the EP was not completely abolished by anoxia. Comparing controls with NMO-treated animals during the 25- to 30-minute period, there were statistically significant differences in the permeability coefficients for Na^+ and Cl^- (Table 2). The permeability ratio of K^+:Na^+:Cl^- was 1:0.09:0.37 in controls and 1:0.04:2.79 in NMO-treated animals. Ion conductances calculated from equation 2 are given in Table 3. Total conductance, which was defined as the sum of each ion conductance, was 0.469 siemens in controls and 0.653 siemens in NMO-treated animals.

DISCUSSION

The present study clarified the following significant differences between control and NMO-treated guinea pigs in the EP, the ion concentrations in inner ear fluid, and the ion permeabilities of the cochlear partition. In NMO-treated animals, 1) the EP was lower than in control animals; 2) the magnitude of the negative EP was smaller than in control animals; 3) the K^+ concentration was decreased and the Na^+ concentration was increased in endolymph, and the K^+ concentration was increased in perilymph; and 4) the permeability of the cochlear partition for Na^+ was lower, and that for Cl^- was higher, than in the controls.

It is widely accepted that the EP is the sum of the electrogenic potential existing in the stria vascularis and the preexisting negative EP. The EP depression induced by NMO can be explained by a decrease in electrogenic potential and/or an increase in the magnitude of passive negative potential. Recently, many investigators[12,14-22] showed that the negative EP is a diffusion potential between perilymph and endolymph, which is closely related with the organ of Corti. The negative EP was consistent with the diffusion potential calculated by the Goldman-Hodgkin-Katz equation using the K^+:Na^+:Cl^- permeability ratio of the cochlear partition and each ion concentration in perilymph and endolymph under anoxic conditions.[12] The permeability ratio of K^+:Na^+:Cl^- (1:0.09:0.37) determined in the present study was almost similar to one (1:0.18:0.23) reported for normal guinea pigs.[12] The negative EP of both control and NMO-treated animals in the present experiment agreed with the calculated diffusion potential (Table 4). This fact suggests that the independent principle of Goldman-Hodgkin-Katz is applicable to the cochlear partition even in NMO-treated animals. Konishi[16] assumed that the slow decline of the EP observed in kanamycin-treated guinea pigs in an early stage of anoxia was attributable to a low K^+ conductance in the organ of Corti. An anoxic alteration of the EP in NMO-treated animals seemed to be brought about by an increase in the Cl^- permeability and a decrease in the Na^+ permeability of the cochlear partition, in addition to the changes in the ion concentrations in inner ear fluid. Assuming that the permeability

TABLE 4. CALCULATION OF DIFFUSION POTENTIAL

Animals	*[K^+]p*	*[K^+]e*	*[Na^+]p*	*[Na^+]e*	*[Cl^-]p*	*[Cl^-]e*	*Calculated Diffusion Potential (mV)*	*Observed Negative EP (mV)*	
30 minutes anoxia									
Control	16.9 (4.4)	137.3 (11.7)	142.0 (7.2)	14.4 (1.2)	130.0 (2.3)	109.3 (2.3)	−26.2	−25.9	
NMO-treated	14.4 (3.6)	104.4 (7.6)	128.9 (8.5)	12.3 (4.5)	128.9 (4.6)	163.1 (18.6)	0.6	− 1.0	
								EP (mV)	*Positive Component (mV)*
Normal									
Control	5.3 (0.7)	157.9 (7.9)	142.4 (6.0)	2.5 (0.4)	113.8 (9.6)	143.8 (8.5)	−27.8	82.4	110.2
NMO-treated	8.2 (1.0)	128.5 (10.6)	117.0 (14.3)	9.6 (3.6)	114.4 (1.2)	140.5 (7.8)	− 2.7	30.8	33.5

Calculated diffusion potential is based on Goldman-Hodgkin-Katz equation. Permeability ratio of K^+:Na^+:Cl^- is 1:0.09:0.37 in controls and 1:0.04:2.79 in NMO-treated animals. Positive component is obtained by subtracting calculated diffusion potential from endocochlear potential (EP). [ion] indicates ion concentration and subscripts p and e denote perilymph and endolymph, respectively. Values in parentheses represent standard error of mean.

NMO — nitrogen mustard-N-oxide.

ratio under anoxic conditions is applicable to the cochlear partition in the normal steady state,[11] the preexisting negative EP is calculated from this permeability ratio and the ion concentrations in perilymph and endolymph. Furthermore, the electrogenic potential (positive component) of the EP, which is derived by subtracting the calculated diffusion potential from the measured EP, is 110.2 mV in control and 33.5 mV in NMO-treated animals (Table 4).

Asakuma et al[3] demonstrated that the electrical resistance of the cochlear partition was not changed by NMO; this is consistent with our finding that there was little change in the total conductance. Ultrastructural observations showed no pathologic changes in the stria vascularis in NMO-treated animals.[2,3] However, the present study reveals the disturbance of the electrogenic potential by NMO. It was reported that NMO was a potent inhibitor of Na^+,K^+-ATPase activity in the cell membrane.[23,24] Therefore, it is considered that Na^+,K^+-ATPase at the basolateral membrane with or without the rheogenic K^+ transport pump at the luminal membrane[15,19,22,25-27] in the marginal cell is blocked metabolically by NMO without causing a histologic change. The affected site(s) may resume normal functioning after a few weeks. The inhibition of the electrogenic mechanism in the stria vascularis by NMO, as well as by Na^+,K^+-ATPase blockers such as ouabain and anoxia,[17,28] is thought to induce the decrease of the K^+ concentration and the elevation of the Na^+ concentration in endolymph. There is a need for supporting experiments involving biochemical or histochemical methods.

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IMMUNOHISTOCHEMICAL ANALYSIS OF THE OLFACTORY MUCOSA BY USE OF ANTIBODIES TO BRAIN PROTEINS AND CYTOKERATIN

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The present study deals with the immunohistochemical detection of four brain-derived proteins and cytokeratin in the normal olfactory mucosa of humans and guinea pigs. Neurofilament protein immunoreactivity was found in the olfactory vesicles, dendrites, and perikaryon of receptor cells, and in thick nerve bundles located deep in the lamina propria. The antiserum to neuron-specific enolase (NSE) selectively stained olfactory receptor cells throughout the length of the bundles. The NSE immunoreactivity also was recognized in nerve bundles of various sizes throughout the lamina propria. Glia-specific S-100 protein immunoreactivity was present in Bowman's glands as well as in all nerve bundles in the lamina propria, but not in any cellular elements constituting the olfactory epithelium. Immunoreactivity for spot-35 protein, which was considered to be specific for cerebellar Purkinje cells, was found in flasklike cells (microvillar cells) occurring near the free surface of the epithelium. The basal cells in the olfactory and respiratory epithelium were stained positively with a cytokeratin antiserum.

KEY WORDS — cytokeratin, immunohistochemistry, neurofilament protein, neuron-specific enolase, olfactory mucosa, S-100 protein, spot-35 protein.

INTRODUCTION

Immunohistochemical techniques have been applied to the morphologic examination of the olfactory mucosa. Olfactory marker protein, originally isolated from the rat olfactory bulb by Margolis,[1] was shown immunohistochemically to occur in the receptor cells of the olfactory epithelium.[2] Olfactory nerves and olfactory glomeruli in the bulbs were also immunoreactive for olfactory marker protein.

On the other hand, we first examined olfactory epithelium by use of antisera against some neuron-specific proteins, and showed that immunoreactivities for neurofilament protein (NFP) and neuron-specific enolase (NSE) were restricted to the primary neurons of the olfactory system in human fetuses.[3] No cellular elements within the olfactory epithelium of human fetuses were reactive to antiserum against glia-specific S-100 protein (S-100).[3] Spot-35 protein (S-35) is another brain protein, which is contained exclusively in cerebellar Purkinje cells.[4] When the olfactory epithelium of guinea pigs was stained with S-35 antiserum, only a small number of receptor cells were immunolabelled.[5] Thus, immunohistochemistry for brain-derived proteins is a useful tool for the morphologic analysis of the olfactory organ. More recently, it has been used for the diagnosis of olfactory disturbance.[6]

The present study deals with immunohistochemical demonstration of NFP, NSE, S-100, S-35, and cytokeratin (CK) in the olfactory mucosa of humans and guinea pigs. The combined immunostaining used in this study makes it possible to discriminate all cellular elements in the olfactory mucosa except the supporting cells.

MATERIALS AND METHODS

Materials. Adult male guinea pigs (body weight, about 400 g) were used. Animals were anesthetized with sodium pentobarbital (Nembutal, 150 to 200 mg/kg intraperitoneally) and killed painlessly. The olfactory and respiratory mucosae were obtained from the superior posterior portion of the nasal septum. Human materials that were assumed to be normal morphologically[7] were obtained by use of a special instrument from two patients with chronic sinusitis and from six fetuses ranging from 16 to 25 weeks' gestation.

Immunohistochemical Staining. The specimens were fixed in Bouin's fluid for 4 to 6 hours, dehydrated in a graded series of ethanol, and embedded in paraffin wax. Paraffin sections were cut at a thickness of 5 µm. Dewaxed sections were processed for the peroxidase-antiperoxidase (PAP) method.

Neurofilament protein antiserum was obtained from a rabbit by injecting an NFP subunit of 145,000 molecular weight purified from rat brain.[8] Neuron-specific enolase antiserum was raised in a rabbit by means of NSE derived from rat brains as the antigen.[9] S-100 antiserum was raised in a rabbit

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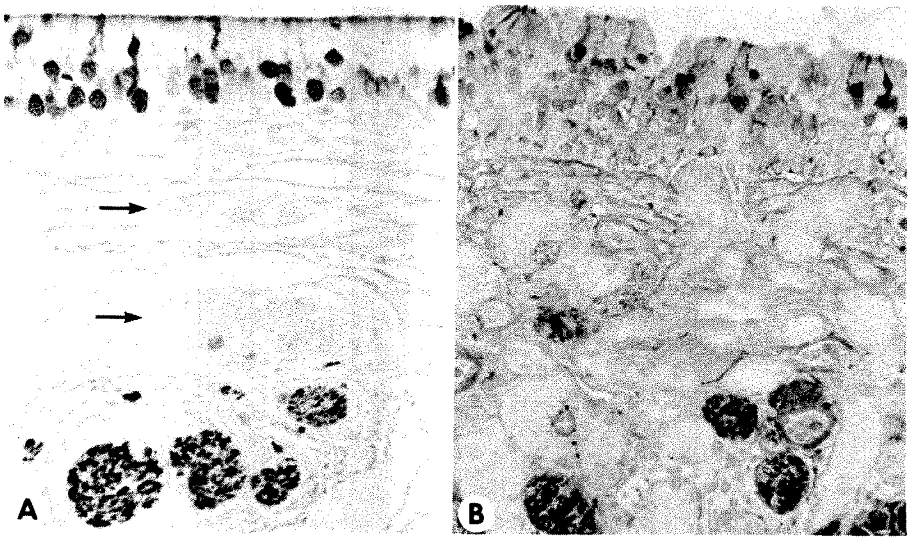

Fig 1. Neurofilament protein immunoreactivity in olfactory mucosa of A) human fetus specimen (original x340), and B) guinea pig specimen (original x400). Immunoreactivity was found in olfactory vesicle, dendrite, and nuclear region of receptor cells, but not in axon of cells. Thick nerve bundles in lamina propria were also immunoreactive for neurofilament protein. Arrows in A — immunonegative nerve bundles from receptor cells.

by injecting S-100 isolated from bovine cerebra.[10] Spot-35 protein was purified from bovine cerebella and the antiserum against it was produced in a rabbit.[4] Cytokeratin antiserum (code A575, DAKO, Santa Barbara, Calif) was raised in a rabbit by use of CK purified from human epidermis as the antigen. Antisera against NFP, NSE, S-100, and S-35 were used at a dilution of 1:1,000, and antiserum against CK was used at a dilution of 1:400.

Conventional staining controls recommended by Sternberger[11] were used to check the specificity of the immunostaining. In addition, the antisera were preincubated with their corresponding antigens (10 to 15 μg/mL of diluted antiserum) for 24 hours at 4°C. The absorbed antisera did not show any immunoreactivity with the olfactory mucosa.

RESULTS

Neurofilament Protein. Immunoreactivity for NFP was found in the receptor cells of both species examined, but not in supporting cells and basal cells. In the receptor cells, the immunoreactivity was restricted to the olfactory vesicles, dendrites, and apical cytoplasm. Their basal cytoplasm and axons were free from the NFP immunoreactivity. Nerve bundles in the lamina propria showed different staining patterns to NFP antiserum; thick nerve bundles running in the lower (deeper) region were stained positively, while thin nerve bundles in the upper region were negative (Fig 1).

Neuron-Specific Enolase. Immunoreactivity for NSE, like that for NFP, was restricted to olfactory receptor cells in humans and guinea pigs. Positive immunoreaction was recognized in their olfactory vesicles, dendrites, and perikaryon. The thin axon of receptor cells was stained positively, but not intensely. The nerve bundles of various sizes distributed widely through the lamina propria showed NSE immunoreactivity (Fig 2).

S-100 Protein. Three cellular elements constituting the olfactory epithelium were all free from S-100 immunoreactivity in both species. Strong immunoreactivity for S-100 was present in the nerve bundles throughout the lamina propria. In the sub-

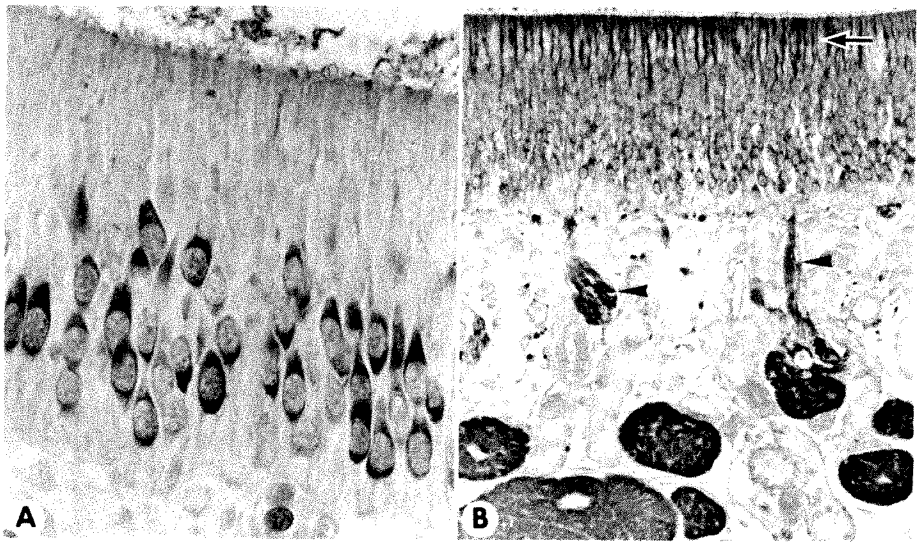

Fig 2. Neuron-specific enolase immunoreactivity in olfactory mucosa. A) Intense immunoreactivity for neuron-specific enolase was recognized in perinuclear cytoplasm of receptor cells in human fetus specimen (original x820). B) Receptor cells in guinea pig specimen displayed immunolabelled dendrites (arrow) (original x420). Neuron-specific enolase immunoreactivity in lamina propria was found in olfactory nerves (arrowheads) as well as nerve bundles running in deeper region.

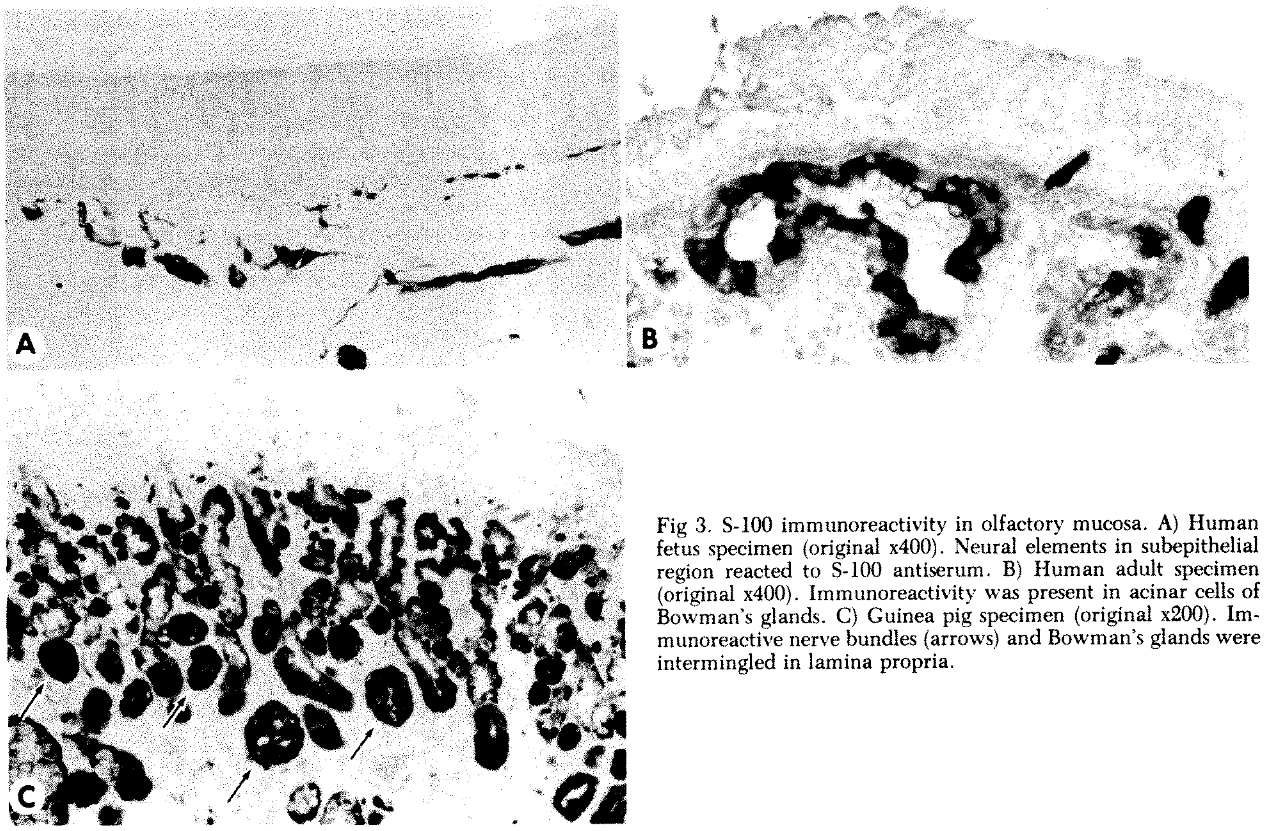

Fig 3. S-100 immunoreactivity in olfactory mucosa. A) Human fetus specimen (original x400). Neural elements in subepithelial region reacted to S-100 antiserum. B) Human adult specimen (original x400). Immunoreactivity was present in acinar cells of Bowman's glands. C) Guinea pig specimen (original x200). Immunoreactive nerve bundles (arrows) and Bowman's glands were intermingled in lamina propria.

epithelial region, Schwann cells that ensheathed the axons of receptor cells were demonstrated clearly. Another positive reaction was found in Bowman's glands. Both acinar cells and intraepithelial ductal cells showed S-100 immunoreactivity. However, no S-100 immunoreactivity was found in the nasal glands and their ducts (Fig 3).

Spot-35 Protein. Flask-shaped cells dispersed near the free surface of the olfactory epithelium reacted intensely to S-35 antiserum in guinea pigs, while in the human olfactory epithelium, no S-35 immunoreactive cells were present. The frequency of occurrence of S-35–positive cells in guinea pigs was considerably low (only two or three in one microscopic field). The apical cytoplasm of the immunoreactive cells reached the free surface of the epithelium; occasional cells extended an axonlike cytoplasmic process toward the basal membrane, but never beyond it (Fig 4).

Cytokeratin. Basal cells, which were round in shape and located on the basal membrane of the olfactory epithelium, were immunoreactive for CK in humans and guinea pigs. At a higher magnification, CK-immunoreactive cells were found to possess an irregular cytoplasm (Fig 5A).

The CK immunoreactivity also was found in the basal cells of the respiratory epithelium (Fig 5B). No immunoreaction for CK was recognized in other cellular elements including the receptor cells, sustentacular cells, and Bowman's glands.

DISCUSSION

In our present study we confirmed in adult humans and guinea pigs our previous finding from immunohistochemical staining in the olfactory mucosa of human fetuses that two neuron-specific proteins, NFP and NSE, are contained selectively in the receptor cells.[3] Neurofilament protein is an important brain protein that forms the neural cytoskeleton. Neuron-specific enolase, a glycolytic enzyme that is present specifically in neurons, develops with the morphologic and functional maturation of neurons.[12] The existence of these neuron-specific proteins in olfactory receptor cells suggests that the receptor cells might possess the same function and quality as authentic neurons. In contrast, Vollrath

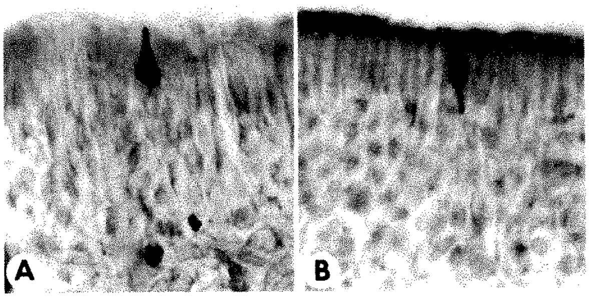

Fig 4. Spot-35 immunoreactivity in olfactory epithelium of guinea pig specimen (original x400). A) Immunoreactivity was restricted to flask-shaped cells near free surface of epithelium. B) Immunoreactive cell extended short process toward basement membrane.

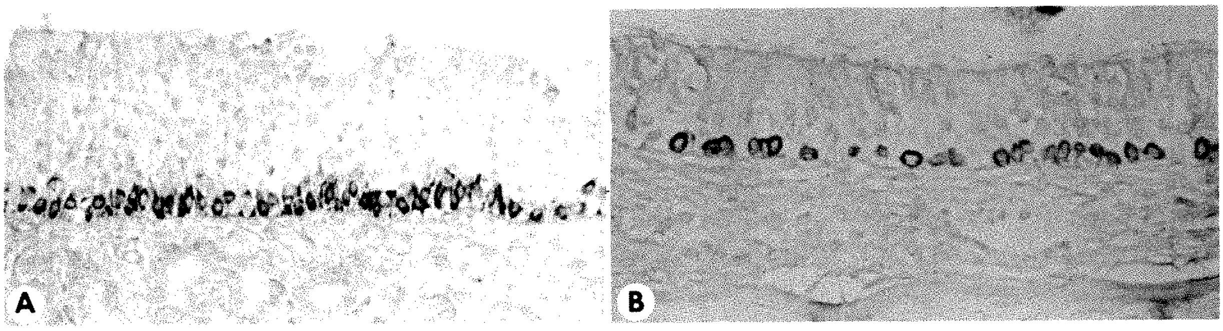

Fig 5. Cytokeratin immunoreactivity in nasal mucosa of guinea pig specimen present in basal cells. A) Olfactory epithelium (original x310). B) Respiratory epithelium (original x400).

et al[13] reported that the olfactory epithelium lacked neurofilament expression, and they had doubts about the neural nature of this tissue.

S-100 is a glia-specific protein contained in astrocytes, oligodendroglia, and ependymal cells in the CNS and in Schwann cells in the peripheral nervous system.[14,15] The S-100 immunoreactivity is shared by sustentacular or supporting cells in the sensory and endocrine organs, where main cells contained NSE immunoreactivity.[16] No supporting cells, however, in the olfactory epithelium of guinea pigs and adult humans were immunoreactive for S-100 as reported in human fetuses.[3] Although the supporting cells have been believed to be of glial type,[17] the present finding suggests that the supporting cells are different from the glial element in function as well as origin. S-100 immunoreactivity was found in this study to occur in Bowman's glands, suggesting that Bowman's gland is derived from a glial element.

Some researchers have observed the fourth cell type, called microvillar cells, which are a minor component in the olfactory epithelium and are regarded as another chemoreceptor.[18,19] Spot-35 immunoreactivity was found only in flask-shaped cells near the free surface of the olfactory epithelium. These cells are similar in distribution and cell shape to microvillar cells. It has been shown that S-35 possesses Ca^{++}-binding ability and that S-35 immunoreactivity is present in some sensory cells, including gustatory cells and hair cells of the inner ear as well as cerebellar Purkinje cells.[20] Ca^{++} is closely related to evoked potentials of the cell membrane and neurotransmission; thus the fact that microvillar cells contain a Ca^{++}-binding protein may support the idea that this cell is a second chemoreceptor.

Intense CK immunoreactivity was restricted to the basal cells of the olfactory and respiratory epithelium. Cytokeratin is a main protein for forming tonofilaments, which are specific for the epithelial cells. This staining result is in agreement with electron microscopic observations that the basal cells are rich in tonofilaments.[21] It is reasonable to presume that these cells have a strong epithelial quality. It generally is accepted that the basal cells in the olfactory epithelium develop into receptor cells, while those in the respiratory epithelium develop into ciliated cells.[22] We can conclude that this type of cell may have a multipotential function.

The lamina propria of olfactory mucosa contains two types of neural elements, derived from olfactory receptor cells and trigeminal ganglions. The olfactory nerves are distributed in the superior portion of the lamina propria, while the trigeminal nerves are distributed in the deep portion. Neuron-specific enolase antiserum was able to reveal both the olfactory and trigeminal nerves. On the other hand, no immunoreactivity for NFP is present in the axons of receptor cells or in olfactory nerve bundles. Thus NFP is a specific marker for the trigeminal nerves in the lamina propria. Olfactory nerves sheathed by Schwann cells react to the S-100 antiserum, from the fine fibers near the basement membrane to thick nerve bundles deep in the lamina propria. It is possible by means of combined immunostaining for NFP, NSE, and S-100 to determine the distribution of olfactory and trigeminal nerves.

We conclude that neuronal and glial proteins and CK can be good markers for examining the morphology and origin of the cellular components, nerves, and glands of the olfactory mucosa.

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EFFECT OF NAPROXEN, A PROSTAGLANDIN INHIBITOR, ON ACUTE OTITIS MEDIA AND PERSISTENCE OF MIDDLE EAR EFFUSION IN CHILDREN

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BENJAMIN M. VOLOVITZ, MD JOSEF E. GROSSMAN, PHD
PETAH-TIQVA, ISRAEL

Prostaglandins are thought to be of importance in the pathophysiology of otitis media with effusion (OME), and the possibility of reducing the frequency and persistence of this condition by using prostaglandin inhibitors has been suggested. In a double blind manner, naproxen was administered to children with acute otitis media, in addition to amoxicillin, and its influence on the subsequent occurrence and persistence of middle ear effusion was evaluated. Eighty-one children participated in the study. No significant difference was found in the number of patients with tympanograms consistent with OME in the two groups. After 10 days of treatment, 63% in the naproxen and 58% in the placebo group, and after 30 days, 41% and 59%, respectively, had type B tympanograms. Similarly, there were no differences between the two groups with respect to other parameters studied (duration of otalgia, fever, otoscopic findings). No side effects related to naproxen were observed.

KEY WORDS — acute otitis media, naproxen, otitis media with effusion, prostaglandins.

INTRODUCTION

Acute otitis media (AOM) is one of the most common problems encountered in everyday pediatric practice. Teele et al[1] reported that one third of children have had three or more episodes of AOM in the first 3 years of life. The acute symptoms of the disease usually are treated successfully by antibiotics; however, middle ear effusion may be present for a long period subsequently. In 40% to 60% of patients, otitis media with effusion (OME) associated with hearing impairment is observed 1 month after an acute episode of otitis media.[2-4] In 20% of patients a hearing loss of 20 dB or more can be demonstrated 6 months following an episode of AOM.[5] The search for more efficient therapy to improve the outcome therefore seems justified.

Anti-inflammatory drugs combined with antibiotics have been shown to have a beneficial effect in the treatment of respiratory infectious diseases, including otitis media in adults[6,7] as well as in children.[8,9] Naproxen is a well-known prostaglandin inhibitor and is used successfully in the treatment of rheumatic conditions in adults[10] as well as in children.[11] In animal studies this drug has been shown to have in addition a prominent anti-exudative effect.[12] Assuming that a similar effect is present in humans, it may be expected that administration of this or a similar preparation could reduce the severity and frequency of exudation associated with AOM. In addition, there is evidence suggesting that prostaglandins E and F may have some role in the pathogenesis of serous otitis media.[13] It can be expected, therefore, that an inhibitor of prostaglandin synthesis such as naproxen may have some effect in the prevention of OME.

In this study we report the results of a double blind clinical trial evaluating the effect of naproxen suspension administered concomitantly with the conventional antibiotic therapy to children with AOM.

PATIENTS AND METHODS

The study included children with clinical symptoms and otoscopically proven AOM, between 1 and 12 years of age, who had been referred to the pediatric emergency room of Beilinson Medical Center and a community pediatric clinic affiliated with it. Patients with chronic or spontaneous eardrum perforation or those requiring tympanocentesis were excluded.

Each patient was examined with a pneumatic otoscope (Heine, Herrsching OBB, Federal Republic of Germany) on entering the study and on the third, tenth, and 30th days. Impedance tympanometry using the Amplaid 709 tympanometer (Amplaid SPA, Milan, Italy) was obtained on days 0, 10, and 30 of the study. A modification of the criteria of Orchik et al[14] was used for interpretation of the tympanograms. Curves with a peak of negative pressure (mm H_2O) of < 100 were classified as type A, those with a peak between 100 and 300 as type C, and flat curves as type B.

The children's parents were asked to record on a

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TABLE 1. DURATION OF SYMPTOMS ASSOCIATED WITH ACUTE OTITIS MEDIA

Study Group	*No. of Patients*	*Temperature ≥38°C (days)*	*Earache (days)*	*Sleep Disturbances (nights)*
Naproxen	24	0.8±1.0	1.25±1.1	0.62±0.87
Placebo	23	0.9±1.1	1.3 ±1.0	1.17±1.5
Total	47			
Significance		NS	NS	NS

Values are mean ± SD.

TABLE 2. OTOSCOPIC FINDINGS ON TENTH DAY OF TREATMENT

Study Group	*No. of Patients*	*Erythema* (%)*	*Bulging* (%)*	*Absence of Light Reflex (%)*
Naproxen	38	41	31	39
Placebo	43	48	26	36
Total	81			
Significance		NS	NS	NS

*Mild to moderate.

diary card during the first 10 days of the study the child's body temperature (thrice daily), earache, sleep disturbances with more crying and wakefulness than usual, and use of any additional drug. At the conclusion of this 10-day treatment period the parents and physician in charge were requested to provide an overall subjective evaluation of the results of treatment, using a scoring scale: 1 — excellent, 2 — good, 3 — fair, 4 — poor, 5 — worse.

The diagnosis of AOM was based on the presence of erythema or whitish yellow opacification accompanied by fullness or bulging, poor mobility of the tympanic membrane, and one or more of the symptoms commonly accompanying acute ear infection, ie, otalgia, irritability, and fever.[15,16] Otitis media with effusion was diagnosed in children recovering from the acute symptoms of AOM in whom the otoscopic signs of acute infection had disappeared and who had a type B tympanogram and impaired eardrum mobility on pneumatoscopic examination.

The study followed a double blind design. Each patient randomly received either naproxen suspension 7 mg/kg three times a day or a placebo. In addition, each patient was given amoxicillin 40 mg/kg/d. All medications were administered for 10 days. The parents were requested to withhold the use of antipyretic and analgesic preparations as much as possible. Patients who received more than one single dose of such medication during the first 10 days of the study period were excluded from the final evaluation. In the emergency room patients, the following laboratory studies were performed on days 0 and 10 to detect any drug toxicity: complete blood count, routine urine analysis, serum electrolytes, proteins, SGOT, LDH, calcium, phosphate, uric acid, alkaline phosphatase, creatinine, and prothrombin.

In cases of bilateral otitis media, the assessment was based on the findings of the ear with the more severe and protracted course.

Compliance was assessed by the ratio of the amount of drug left in the bottles returned at the visit on the tenth day as compared to the amount prescribed.

The study was approved by the hospital's ethics committee. Informed written consent was obtained from one of the parents prior to inclusion of each patient in the study.

The statistical evaluation employed the χ^2 and Student's *t* test.

RESULTS

One hundred one patients initially were included in the study, 81 of whom were followed for 10 days (41 boys and 40 girls, 1 to 12 years of age, mean age of 3¼ years). Seventy-six were available for examination on the 30th day of the study. Fifty-three of the 81 subjects were from the community clinic and 28 from the hospital emergency room. Of the 20 patients not included in the 10-day evaluation, nine were in the naproxen and 11 in the placebo group. The main reasons for withdrawal and exclusion were refusal to come to follow-up visits (eight in each group), use of acetaminophen on more than one occasion (one in each group), severe intercurrent disease (gastroenteritis; one in the placebo group), and spontaneous perforation of the eardrum (one in the placebo group). Of the five additional subjects lost to follow-up on the 30-day visit, three were from the naproxen and two from the placebo group. According to available data, the demographic and clinical profiles are similar in those patients who were excluded or who withdrew from the study to those in patients who remained for final evaluation.

Thirty-eight children received naproxen suspension (25 from the community clinic and 13 from the emergency room), and 43 received placebo. The mean age in the two groups was 3 years 11 months and 2 years 9 months respectively.

Relevant data from the diary cards were available from 47 patients, 24 in the naproxen group and 23 in the placebo group. No difference was found between the two groups in relation to the number of days of fever ≥ 38° C, earache, or sleep disturbances (Table 1). Likewise, no difference was found in the otoscopic signs on conclusion of the 10-day treatment period (Table 2). The scoring of the evaluation of the overall efficacy of the treatment was similar for the naproxen- and placebo-treated patients, 2.0 and 2.36 respectively by the physician in charge, and 2.10 and 2.18 by the parents.

The proportion of patients with type B tympanograms on day 10 was almost identical in the patients receiving naproxen or placebo. On the 30th

TABLE 3. TYPES OF TYMPANOGRAMS

	10 Days				*30 Days*			
	Patients Examined	*Patients and % With*			*Patients Examined*	*Patients and % With*		
Study Group	*No. (%)*	*Type A*	*Type C*	*Type B*	*No. (%)*	*Type A*	*Type C*	*Type B*
Naproxen	38 (100)	4 (11)	10 (26)	24 (63)	34 (100)	9 (27)	11 (32)	14 (41)
Placebo	43 (100)	7 (16)	11 (26)	25 (58)	42 (100)	7 (17)	10 (24)	25 (59)
Total	81	11	21	59	76	16	21	39
Significance			NS	NS			NS	NS

Type A tympanogram — curve with peak negative pressure of < 100 mm H_2O, type C — curve with peak between 100 and 300 mm H_2O, type B — flat curve.

day, however, the proportion of patients with tympanographic evidence of OME was found to be smaller in the naproxen than in the placebo group, 41% and 59% respectively. This difference, however, was not statistically significant ($X^2 = 2.59$) (Table 3). A similar trend also was observed for the subjects with type C tympanograms. According to the reports from the diary cards available (47 patients), no side effects related to the drugs used were observed. Laboratory studies were performed on 27 of the patients seen in the hospital emergency room, 12 in the naproxen and 15 in the placebo group. No evidence of toxicity was found in any of these patients (Table 4).

Compliance in the administration of anti-inflammatory medication was 87% for the naproxen group and 84% for the placebo group, and for amoxicillin therapy it was 89% and 88% respectively.

Although the use of antipyretics was discouraged, ten patients (26%) in the naproxen group and nine (21%) in the placebo group received a single dose of acetaminophen.

DISCUSSION

Prostaglandins are released by a host of mechanical, chemical, bacterial, and other insults and are believed to contribute greatly to inflammation, particularly in more slowly developing and persistent processes[17,18] such as most probably take place in OME. Bernstein et al[13] have demonstrated that in patients with middle ear effusion, prostaglandin E and F levels in the effusion fluid were higher than in the patient's sera. In the middle ear effusions from experimentally induced serous and purulent otitis media in chinchillas, Jung et al[19] found elevated prostaglandin levels, particularly prostaglandin E2, suggesting that this substance may play a role in inflammation and increased vascular permeability of the middle ear mucosa.

The demonstrated results showed that naproxen as used in this trial had no effect on the course of the acute symptoms of otitis media. The effect of antibiotic therapy seems to be so prominent that further symptomatic improvement cannot be expected by additional treatment.

We were unable to demonstrate a statistically sig-

TABLE 4. LABORATORY DATA IN CHILDREN WITH ACUTE OTITIS MEDIA TREATED BY COADMINISTRATION OF AMOXICILLIN AND NAPROXEN

	Amoxicillin and Naproxen (n = 12)					*Amoxicillin and Placebo (n = 15)*				
	Before Treatment		*10 Days With Treatment*			*Before Treatment*		*10 Days With Treatment*		
Laboratory Test	*Mean*	*No.*	*Mean*	*No.*	*Difference*	*Mean*	*No.*	*Mean*	*No.*	*Difference*
Hemoglobin g/dL	11.7	12	11.4	12	− 0.3	11.5	15	11.7	15	− 0.2
WBC x $10^3/mm^3$	9.3	12	9.2	12	− 0.1	10.1	15	10.6	15	0.5
Platelets $10^3/mm^3$	275.0	6	263.0	4	−12.0	312.0	9	322.0	8	10.0
SGOT U/L	45.8	10	48.1	11	2.3	41.1	15	48.0	15	6.9
LDH U/L	250.0	12	251.0	12	1.0	277.0	15	275.0	15	− 2.0
Alkaline phosphatase U/L	187.0	12	202.0	12	15.0	198.0	14	220.0	15	22.0
Uric acid mg%	3.9	12	4.0	12	0.1	4.5	14	4.4	14	− 0.1
Creatinine mg/dL	0.6	12	0.5	12	− 0.1	0.6	13	0.6	15	0.0
Phosphorus mg/dL	4.8	12	4.7	12	− 0.1	5.1	14	5.1	15	0.0
Calcium mg/dL	9.7	12	9.8	12	0.1	9.1	15	9.9	15	0.8
Albumin g/dL	4.2	12	4.2	12	0.0	4.4	14	4.3	15	− 0.1
Total protein g/dL	6.9	11	6.8	12	− 0.1	7.0	14	7.0	15	0.0
Chloride mEq/L	99.5	12	98.1	11	− 1.4	103.0	14	104.0	15	0.1
Potassium mEq/L	4.5	12	4.6	12	0.1	4.5	14	4.7	15	0.2
Sodium mEq/L	137.0	12	138.0	11	1.0	138.0	14	138.0	15	0.0
BUN mg/dL	7.8	5	11.4	6	3.6	9.7	9	10.3	9	0.6
Prothrombin time %	90.0	5	86.0	5	4.0	86.0	10	87.0	9	1.0
Urine	Normal	11	Normal	4		Normal	13	Normal	13	

No. — number of patients examined.

nificant effect of naproxen on the occurrence and persistence of subsequent middle ear effusion. There is, however, a prominent trend in patients receiving naproxen to have less OME 30 days after an AOM episode. In order to demonstrate the potency of this trend, evaluation of a larger study population would probably be of value. However, it is very unlikely that the effect of an anti-inflammatory medication such as naproxen would be evident late in the course of the disease, when no effect was observed at the end of the treatment period.

A more complete understanding of the sequence of events in the pathogenesis of the inflammatory process of the middle ear is needed for interpretation of the observed results. It is possible that the role of prostaglandins, although present, is not critical to the pathogenesis of middle ear effusion. Alternatively, naproxen, at least as used in this trial, may not be adequate for inhibition of the prostaglandin effect on the inflammation of the middle ear.

Recently Jung et al[20] have shown that ibuprofen combined with penicillin for treatment of experimental pneumococcal otitis media was associated with more mucoperiosteal histopathology than was observed in animals treated with penicillin alone. It has been suggested that the inflammation of the middle ear mucosa involves several mediators acting either in sequence or independently.[21] Blocking of each of these mediators separately may therefore be needed.[22] To achieve this, a combination of drugs may be necessary.

Further studies using other prostaglandin inhibitors alone or in combination over longer periods are necessary for clarification of the therapeutic value of these drugs in otitis media. Since there is such a multitude of variables acting on the pathogenesis of OME following an episode of AOM, administration of prostaglandin inhibitors to patients with OME after recovery from the acute episode may be a more suitable line of investigation.

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PRIMARY INTRAVESTIBULAR LIPOMA

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An intravestibular lipoma was discovered during destructive surgery for disabling vertigo in a case diagnosed as delayed endolymphatic hydrops. The author recommends that in cases that resemble Meniere's disease but have unusual symptoms, the existence of other lesions within the inner ear should be suspected.

KEY WORDS — intravestibular lipoma.

INTRODUCTION

Intracranial lipomas are rare tumors that often are associated with cerebral abnormalities and generally are congenital. Such tumors occur most commonly in the corpus callosum, and the rarest location is the cerebellopontine (CP) angle.[1-5]

This report presents the first recorded case of an intravestibular lipoma. Although the tumor was found accidentally during destructive surgery for what had been diagnosed as delayed endolymphatic hydrops, the author suggests that the lipoma in fact was not coincidental to the unusual clinical condition encountered.

CASE REPORT

A 35-year-old woman had suffered from high frequency tinnitus, fullness in the right ear, and episodic vertigo for more than 10 years; she was unable to remember which started first. In the early stages the vertigo occurred only every 1 to 2 years. Gradually it increased in frequency until in the 3 months before surgery it had become disabling, with episodes almost daily and nausea and vomiting particularly in the mornings. Between episodes there was no unsteadiness; however, the patient experienced fullness of the occipital region and the right ear.

Upon presentation on Oct 18, 1986, audiometric examinations revealed normal hearing on the left, whereas there was no response in the right ear to the maximum limits of the audiometer at all frequencies. The patient had not been aware of the loss of hearing, although she had had mumps in early childhood and afterward tended to use her left ear more to hear. A speech discrimination test revealed a score of 100% in the left ear and 0% on the right.

A thorough workup was completed, and results of blood tests including thyroid function, STS, and other routine blood examinations proved normal. Radiography that incorporated mastoid x-ray studies of Towne's and Stenvers' views and polytomography of the internal auditory canals revealed the usual pneumatization of both mastoids and normal caliber of both internal auditory canals. Examination of the cochlea and vestibule and computed tomography also had essentially unremarkable findings. Studies of the vestibular aqueduct, meanwhile, showed regular configuration of the vestibular aqueduct on the right side, but it could not be seen on the left. Electronystagmographic examinations with caloric air and ice water tests resulted in no response on the right and normal findings on the left. The case was diagnosed as probable delayed endolymphatic hydrops, and because of disabling vertigo that failed to respond to medical treatment, destructive surgery in the form of a transmastoid labyrinthectomy was performed on the right ear on Dec 12, 1986.

Upon opening the bony labyrinth into the vestibule, a small solid tumor was discovered in the region of the superoposterior angle of the intact medial wall of the vestibule, but the remainder of the vestibule was totally free of tumors (Fig 1A). The tumor was a soft, oval-shaped, yellowish-brown smooth mass of 2 x 2 x 2.5 mm in size (Fig 1B). Findings from histopathologic studies revealed a tumor composed of mature adipose tissue consistent with a lipoma surrounded by vestibular epithelium (Fig 2).

Immediately after operation there was a decrease in tinnitus, minor fullness of the occipital region, and spontaneous nystagmus toward the healthy side, although only for a few days. Ultimately there was a rapid and smooth complete recovery, and 1 week after operation the patient was free of all symptoms.

DISCUSSION

Intracranial lipomas are rare tumors of a mesodermal origin that usually coexist with malfor-

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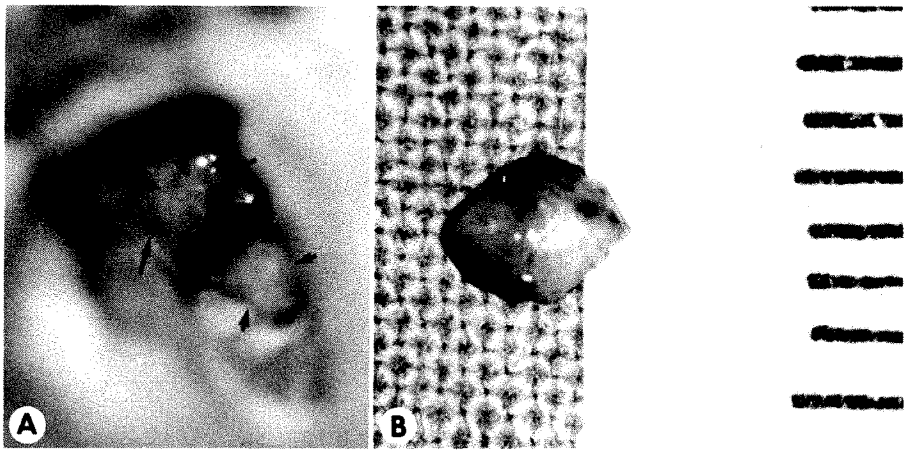

Fig 1. Intravestibular lipoma. A) Photograph taken during transmastoid labyrinthectomy showing tumor (short arrows) occupying superoposterior part of vestibule (long arrows). B) Size and appearance of tumor.

mations and occur predominantly in the corpus callosum, situated in the midline of the brain.[6] The discovery of such tumors in the CP angle is particularly uncommon; indeed, a total of only eight surgical cases have been reported in the world literature.[1-5]

Lipomas in the CP angle differ slightly from intracranial lipomas in that they normally are not associated with malformations, whereas their symptoms indicate clinical characteristics indistinguishable from those of a small acoustic neuroma.[1-5] Diagnostically, CT has become an invaluable tool in identifying these two types of tumor because a CP angle lipoma, unlike a small acoustic neuroma, normally reveals itself as a hypodense mass without enhancement.[3,5] A further difference encountered during surgery is that CP angle lipomas, like other intracranial lipomas, often are incorporated within the neurovascular band, and their removal frequently jeopardizes facial or cochlear functions as well as surrounding brain structures.

This particular case, to my knowledge, is the first intravestibular lipoma ever described in the medical literature. Preoperatively the case was diagnosed as delayed endolymphatic hydrops, although there were unusual clinical characteristics. Even though there was total loss of the vestibular function, there was disabling vertigo that ultimately necessitated total vestibular ablation. It would seem logical to assume that the cochlear symptoms were attributable to mumps labyrinthitis that the patient suffered in early childhood prior to the onset of the hearing loss. This does not, however, explain the vestibular symptoms, because mumps labyrinthitis

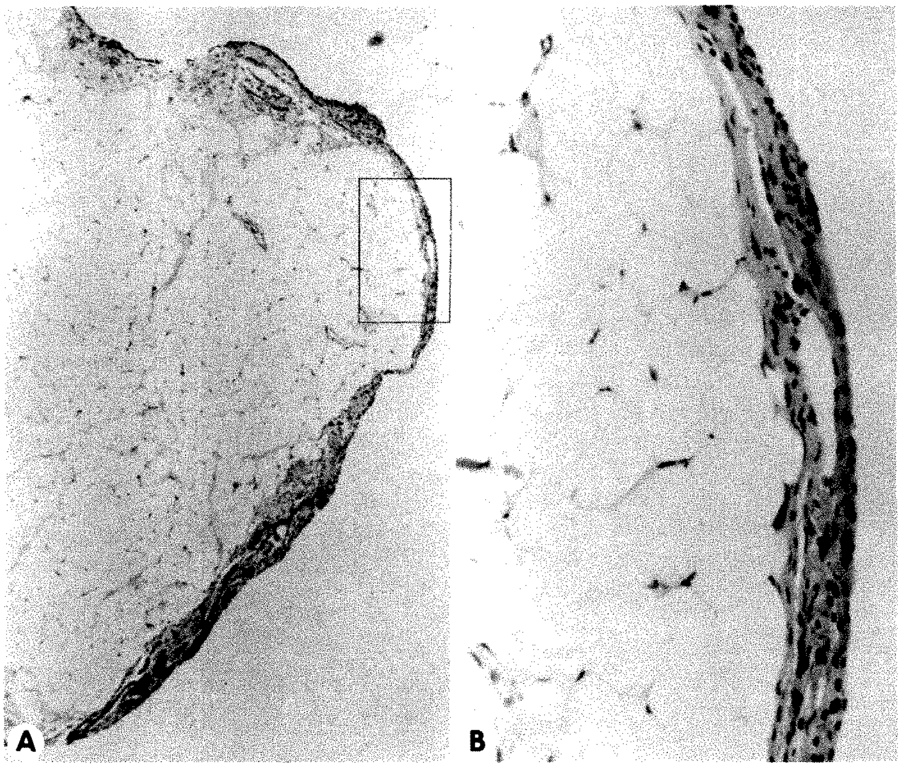

Fig 2. Photomicrographs of section of tumor. A) Mature adipose cells surrounded by vestibular epithelium (H & E, x40). B) Higher power view (H & E, x400).

usually causes a unilateral severe hearing loss and spares the vestibular function.

It is suggested that these symptoms can be explained by the original diagnosis of delayed endolymphatic hydrops, which could have developed following mumps labyrinthitis. Thus the tumor accidentally discovered during surgery could be unrelated to the symptoms encountered. Considering the location of the tumor, however, it would be equally rational to argue that the lipoma may have compressed the proximal endolymphatic system such as the utricular duct or sinus of the endolymphatic duct. The flow of endolymph to the sac, where it is assumed to be resorbed, thereby would have been obstructed; this obstruction would cause endolymphatic hydrops, mimic the symptoms of Meniere's disease, and produce gradual deterioration of the vestibular function over a period of about 10 years.

Therefore it can be seen that while the tumor was an accidental discovery, it was potentially far from coincidental to the symptoms. Indeed, the aforementioned hypothesis logically explains the contradiction between 1) a lack of response to the ice water caloric test similar to that of the burnt-out stage of Meniere's disease and 2) an early loss of hearing following mumps and disabling vertigo.

In this context it is particularly relevant to note that the clinical picture of this case was similar to that of the intralabyrinthine schwannoma I previously reported.[7] I would suggest therefore that in cases in which there appears to be a burnt-out stage of Meniere's disease but the patient displays unusual symptoms of episodic vertigo, the physician should suspect the existence of lesions within the inner ear other than those of Meniere's disease.

Furthermore, according to this author's experience, a labyrinthectomy is the only appropriate procedure when hearing is not worth preserving and there is a suspicion of a tumor such as an intralabyrinthine schwannoma or an intravestibular lipoma (as in this case). In cases in which there is profound loss of vestibular function, labyrinthectomy usually provides rapid and complete symptom relief.

This is the first reported case of an intravestibular lipoma, unique in that there was a history of mumps labyrinthitis and a symptom complex mimicking Meniere's disease. It is my recommendation that in cases resembling the end stages of Meniere's disease in which there is an unusual clinical picture other lesions be suspected within the inner ear and a labyrinthectomy be performed in preference to other therapeutic modalities.

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IMAGING CASE STUDY OF THE MONTH

EXTRACRANIAL CAROTID ARTERY ANEURYSM: AN UNUSUAL NECK MASS IN A YOUNG ADULT

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INTRODUCTION

The spontaneous occurrence of an extracranial carotid artery aneurysm in a healthy 19-year-old woman with no history of cardiovascular disease or cervical trauma is unusual. Aneurysm formation secondary to spontaneous extracranial carotid artery dissection has been described in the vascular and neuroradiologic literature. Otolaryngologists need to consider this entity as a possible cause of a neck mass even in a young individual.

CASE REPORT

A 19-year-old woman presented to her obstetrician 1 month postpartum with a left-sided neck mass of recent onset. She described an associated dull, aching pain radiating into the left jaw and aural regions. She denied neurologic or visual symptoms. There was some question of a low-grade fever. Oral antibiotics were prescribed, but the mass failed to resolve and otolaryngologic consultation was requested.

Her pregnancy had been complicated only by placenta previa necessitating delivery by caesarean section without active labor. There had been no postpartum difficulties. She had no history of hypertension or cardiovascular disease. She denied cervical trauma. Physical examination revealed a healthy-appearing, normotensive young woman. A 3.0 x 4.0-cm, nontender, firm mass was palpable in the left superior cervical region immediately inferior to the angle of the mandible. A slight pulsatile characteristic to the mass was noted. There was no associated lymphadenopathy. Findings on oral and nasopharyngeal examinations were entirely within normal limits, as were those of neurologic and systemic examinations. A computed tomographic scan with contrast enhancement confirmed the presence of a 2.0 x 3.5 x 4.0-cm mass within the left parapharyngeal space deep to the sternocleidomastoid muscle (Fig 1). The mass appeared well-defined and was markedly enhanced.

Needle biopsy was deferred. The left side of the neck was explored with the patient under general anesthesia. Operative examination revealed a pulsatile mass arising from the internal carotid artery. No biopsy of the mass was undertaken.

Bilateral carotid angiography was performed (Fig 2). A large aneurysmal dilatation of the left internal carotid artery was documented 5.0 mm distal to the carotid bifurcation; an additional, smaller dilatation was present more superiorly at the base of the skull. No "string of beads" sign suggestive of

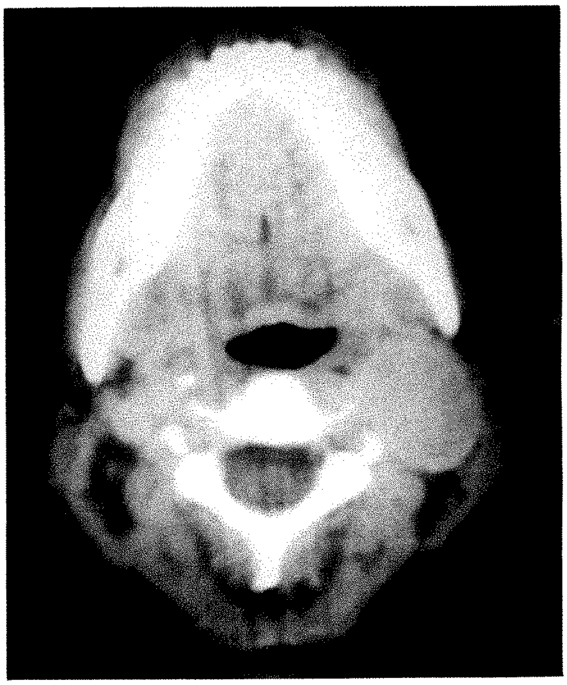

Fig 1. Axial contrast computed tomogram at level of mandibular angle showing well-defined, rounded mass deep to left sternocleidomastoid muscle abutting on parapharyngeal space. Jugular vein and internal carotid artery cannot be distinguished from mass.

From the Department of Otolaryngology (Cunningham), University of Pittsburgh Eye and Ear Hospital, and the Departments of Otolaryngology (Rueger) and Radiology (Rothfus), Allegheny General Hospital, Pittsburgh. Dr Cunningham is now at the Massachusetts Eye and Ear Infirmary, Boston.

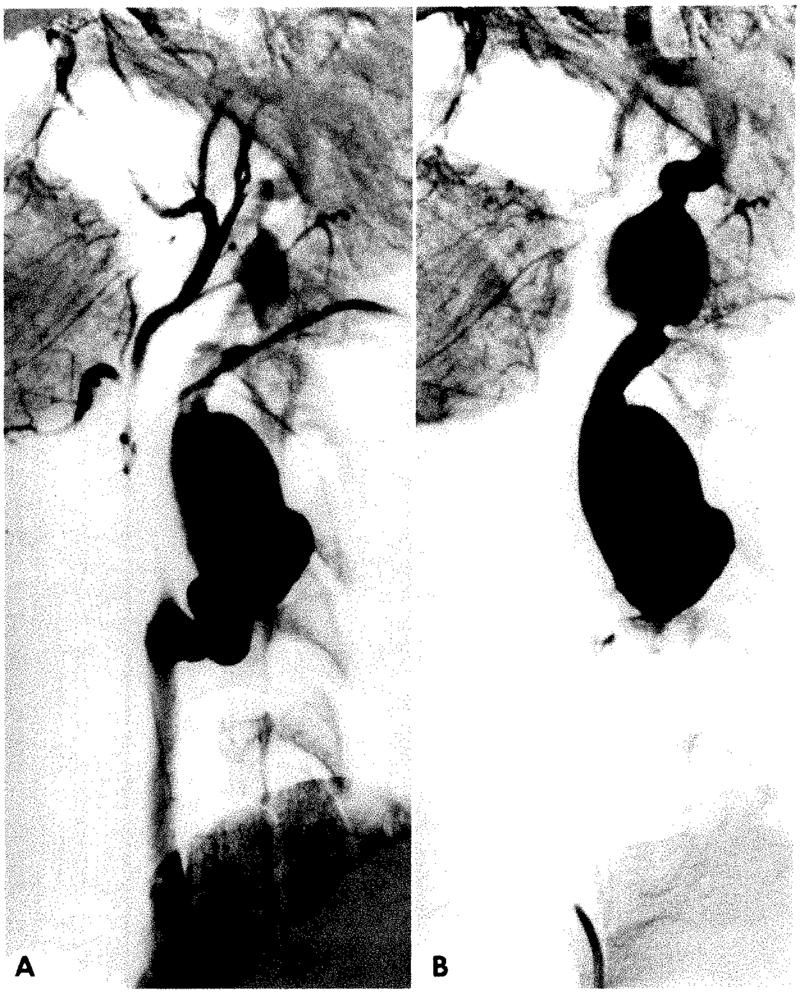

Fig 2. Lateral subtraction views of left common carotid angiogram demonstrating irregular, aneurysmal dilatation of cervical internal carotid artery, with normal common carotid artery and normal intracranial internal carotid artery. A) Early view, B) midphase view.

fibromuscular dysplasia was noted. The right carotid arterial system was normal. Neurosurgical consultation was requested. The neurosurgeon felt that the location of the aneurysms precluded carotid resection with interpositional grafting. Embolic prophylaxis with aspirin therapy was prescribed.

Despite treatment, the patient began to experience slight visual changes suggestive of emboli. Angiography was repeated with balloon occlusion of the left common carotid artery; occlusion was tolerated for 20 minutes with no evidence of neurologic symptoms. These results implied the patient was a suitable candidate for proximal carotid ligation. Exploration of the neck with application of a Crutchfield clamp to the left common carotid artery was performed. Perioperative anticoagulation therapy was employed. The patient experienced no neurologic complications. She remains asymptomatic 28 months postoperatively.

DISCUSSION

Aneurysms of the extracranial carotid arteries are quite uncommon in comparison to the incidence of intracranial carotid aneurysms and of aneurysms arising elsewhere in the arterial system.[1] The common and internal carotid arteries are the usual extracranial sites of aneurysm origin; the external carotid artery rarely is involved.[2] Syphilis and oropharyngeal streptococcal infection were the common causes in the past. Most patients with extracranial carotid aneurysms today have a history of long-standing atherosclerosis, predisposing cervical trauma, or previous carotid surgery.[3] An uncommon cause of extracranial carotid aneurysm formation is atraumatic arterial wall dissection.[4-6] Many of these spontaneous dissection aneurysms occur in patients with systemic angiopathic disorders such as fibromuscular dysplasia, cystic medial necrosis, or Marfan's syndrome. Fibromuscular dysplasia, in particular, demonstrates a predilection for the carotid vessels.[7]

Rarely, extracranial carotid aneurysms, both with and without associated arterial dissection, have been reported in patients with no predisposing history, physical findings, or angiopathic changes.[3,6,8] Unilateral, single vessel involvement is the rule in these cases in contrast to systemic, bilateral vessel disease. Such patients are also characteristically young to middle-aged adults, whereas atheromatous disease and fibromuscular dysplasia typically present in the 40- to 70-year age group.[9]

Extracranial carotid artery aneurysms, regardless of cause, typically present as a superior cervical neck mass. Occasionally there is an accompanying intraoral parapharyngeal mass presentation.[10] The mass may or may not be tender; it is usually pulsatile. Unilateral headache and cephalad radiation of neck pain are particularly characteristic of dissection aneurysms.[4,6] Transient and permanent neurologic and visual deficits may occur secondary to emboli and thrombus formation.[5,11] Peripheral cranial nerve palsies and Horner's syndrome reflect direct nerve compression by the expanding aneurysm.[12] Dysphagia may occur in patients with an intraoral parapharyngeal mass presentation. Spontaneous hemorrhage is rare.[13]

An extracranial carotid artery aneurysm must be distinguished from other lesions known to arise within the parapharyngeal space of the cervical neck such as paragangliomas, neuromas, neurofibromas, lipomas, lymphomas, and nodal metastases.[14] Branchial cleft cysts may also present in the region.

Computed tomography using rapid bolus contrast injection has replaced ultrasonography as the noninvasive modality of choice in cervical neck mass evaluation.[14] This CT protocol can often delineate carotid anatomy sufficiently to enable the diagnosis of an extracranial carotid aneurysm.[15,16] The potential role of magnetic resonance imaging in this regard is undetermined at present.

Arteriography remains the gold standard for establishing the diagnosis of extracranial carotid arterial aneurysm.[3,10,17] The masquerading aberrant or tortuous carotid vessel is easily ruled out, and etiologic cues—for example, the "string of beads" sign representing the segmental vessel constrictions and dilatations characteristic of fibromuscular dysplasia—may be apparent. From a therapeutic standpoint, arteriography is useful in defining both the anatomic extent of the aneurysm and collateral, particularly cerebral, flow patterns.

There is no controversy as to the need for surgical intervention in symptomatic extracranial carotid artery aneurysms. Asymptomatic extracranial aneurysms, particularly high internal carotid lesions, pose a therapeutic dilemma.[3] Treatment options include observation, an attempt at surgical repair, or ligation of the carotid artery proximal to the aneurysm.

Conservative observation of asymptomatic extracranial carotid aneurysms is problematic because of the expectation of ongoing degeneration and the increasing likelihood of neurologic sequelae.[3] Atraumatic dissection aneurysms may be an exception given the observed tendency of these particular aneurysms to diminish or resolve spontaneously with time.[6] Anticoagulation with heparin sulfate or a fibrinolytic or antiplatelet agent is the treatment of choice when surgical management is not planned initially.[18] Direct resection of extracranial carotid aneurysms with reestablishment of arterial continuity is the most desirable surgical approach.[10,17] Technical options include aneurysmectomy with a patch graft, an interpositional vein or Dacron graft, or an anastomosis between the external and distal carotid stump.

Distal control to allow direct resection of high extracranial internal carotid aneurysms cannot always be accomplished. Proximal ligation of the carotid, typically by means of a Crutchfield or Selverstone artery clamp, is the surgical alternative.[3] In such cases an accurate preoperative estimation of carotid stump pressure and intracranial collateral flow is necessary before proximal carotid ligation is performed. Systolic carotid stump pressure in excess of 70 mm Hg traditionally has been considered indicative of adequate collateral blood flow.[19] This determination can be made noninvasively with reasonable accuracy by ocular pneumoplethysmography performed during digital occlusion of the common carotid artery.[20] These methods have been supplanted recently by balloon occlusion and xenon flow studies, which more accurately predict those patients in whom relatively safe ligation of the carotid artery is possible and those at high risk for intracranial neurovascular complications.[21,22]

Hypotension, anemia, and carotid sinus reflex responses are important causes of immediate neurologic complications following carotid artery ligation.[15] Distal thrombosis of the ligated vessel with subsequent embolization and infarction are worrisome late sequelae. Postoperative maintenance of sustained arterial pressure and blood volume is essential in avoiding immediate problems. Long-term anticoagulation is advocated to prevent delayed complications.

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Ann Otol Rhinol Laryngol 98:1989

PATHOLOGY CONSULTATION

EWING'S SARCOMA OF BONE AND SOFT TISSUES

NOUR SNEIGE, MD JOHN G. BATSAKIS, MD

HOUSTON, TEXAS

Ewing's sarcoma is a distinctive clinical and pathologic entity that may be intraosseous or of soft-tissue origin. In the facial bones, the incidence of the sarcoma is estimated at 2.5% of all Ewing's sarcoma of bone. The soft tissues of the head and neck account for 11% of extraskeletal sites of the neoplasm. Combined modality treatment has modified its high-grade malignancy.

The histogenesis of Ewing's sarcoma remains unknown although there is considerable evidence to support origin from undifferentiated mesenchymal cells.[1,2] The neoplasm exists in skeletal and extraskeletal forms without any significant differences in appearance at either light or electron-optic levels of examination.[3,4]

In bone, Ewing's sarcoma accounts for 5% to 10% of all malignant tumors. The pelvic girdle and long bones of the lower limbs are the favored sites of origin. The neoplasm ranks second to osteosarcoma as the most common osseous malignancy in the second decade of life. Involvement of the gnathic bones is considerably less. By 1983, 86 variably documented cases of Ewing's sarcoma of the jaws had been reported.[5] In facial bones and the calvarium, the incidence of Ewing's sarcoma has been estimated at 2.5% and 0.7%, respectively.[6]

The Table, modified from Stuart-Harris et al,[7] gives the primary site of 136 cases of extraskeletal Ewing's sarcoma. The head and neck area ranks with the chest wall in frequency behind extremities and paravertebral/epidural locations.

Clinicopathologic features of Ewing's sarcoma of the jaws do not differ from those associated with the tumors occurring at other bony sites. The patients are young at time of diagnosis (average age of 15 years with a range of 3 to 38 years). There is a male preponderance. Pain is the most common presenting symptom, usually becoming increasingly severe and persistent. Fever, anemia, and leukocytosis also may be present. Som et al[8] note that patients without systemic findings appear to have a longer survival. Very often the signs and symptoms are attributed erroneously to dental disorders until the neoplasm becomes evident.

The mandible is affected nearly three times more often than the maxilla. In the mandible, the ramus, angle, and posterior body have been most often the areas of involvement. The temporal bones, ethmoids, and turbinates are also reported sites.[5]

Radiographically, Ewing's sarcoma has been called a "great imitator" since it can be confused with almost any benign or malignant lesion of bone. The jaw and peripheral skeleton lesions, however, present similar radiographic features. There is a permeative (mottled osteolytic) destruction of bone with cortical expansion and soft tissue swelling. The periosteal reaction can be lamellated or spiculated. Because of the complex anatomy, an "onion-skin" reaction is demonstrated unusually in the facial bones. The incidence (90%) and characteristics of the adjacent soft tissue mass in the mandible are similar to those in the peripheral skeleton.[9]

A diagnosis of Ewing's sarcoma of bone usually can be made without much difficulty when an adequate biopsy sample is provided. Location of the tumor, age of the patient, and radiographic findings, together with the light microscopic findings of closely packed, small, relatively uniform, glycogen-rich round cells, are nearly diagnostic. In metastatic foci or in extraskeletal tumors, Ewing's sarcoma

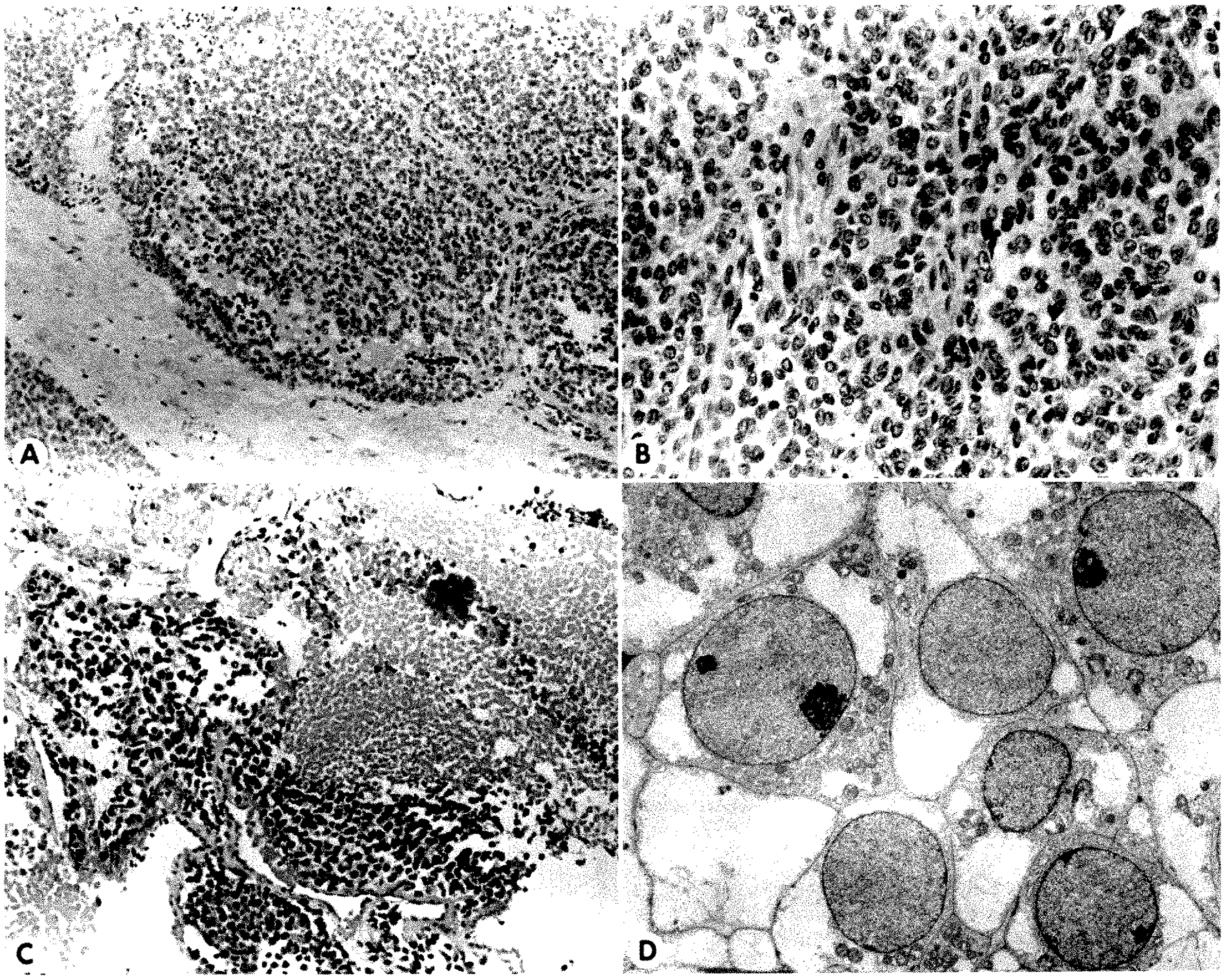
EXTRASKELETAL EWING'S SARCOMA IN 136 PATIENTS

Site	*No. of Patients*	*Percent*
Extremity	44	32
Paravertebral/epidural	21	15
Head and neck	15	11
Chest wall	15	11
Pelvis	9	7
Retroperitoneum	8	6
Buttocks	6	4
Axilla	6	4
Shoulder	3	2
Mediastinum	3	2
Perineum/scrotum	2	1
Groin	1	1
Hip	1	1
Lung	1	1
Abdominal wall	1	1

From data presented by Stuart-Harris et al.[7]

From The University of Texas M. D. Anderson Cancer Center, Houston.

REPRINTS — John G. Batsakis, MD, Division of Pathology, The University of Texas M. D. Anderson Cancer Center, 1515 Holcombe Blvd, Houston, TX 77030.



Ewing's sarcoma of bone. A) Uniform cell population in lobular architecture (H & E, original x50). B) Scattered, distorted cells with hyperchromatic nuclei in midst of typical Ewing's sarcoma cells (H & E, original x130). C) Coagulative necrosis of nearly one half of neoplasm in field (H & E, original x80). Note perithelial aggregation of viable cells. D) Electron-optic appearance (original x4,300). Note uniform nuclei, closely apposed cells without junctions, and clear zones of glycogen.

must be distinguished from other histologically similar malignancies, including neuroblastoma, rhabdomyosarcoma, and lymphoma.

The foregoing brief description of the histologic appearance of Ewing's sarcoma is too simplistic, both in growth pattern and cytomorphology. There are several architectural arrangements of the sarcoma: diffuse fields or sheets of cells (most common), lobular with cellular aggregates separated by fibrovascular septae (see Figure, A), and a less common organoid pattern. Although moderate-sized round to oval cells are the preponderant cells, there is a dimorphic cell population with smaller, less uniform eosinophilic cells with dense, hyperchromatic nuclei (see Figure, B). These cells occur in a random or perilobular distribution and are regarded as degenerated or effete tumor cells. Necrosis, hemorrhagic or coagulative, may be localized or extensive (see Figure, C).

The histopathologic spectrum of Ewing's sarcoma is further broadened by the existence of an atypical or large-cell variant.[10,11] This variant may be of bone origin or be extraskeletal and manifests either an immature mesenchymal or histiocytic appearance. In the typical Ewing's sarcoma, mitoses and nucleoli are rarely prominent. In contrast, the nuclei of the large-cell Ewing's sarcoma are nearly twice as large, manifest considerable irregularity in form, and frequently show prominent nucleoli and numerous mitoses. No clinical or anatomic differences appear to exist between the variants.

The cytologic differences between typical and atypical Ewing's sarcoma are also carried over to their ultrastructure (see Figure, D). In the former, undifferentiated cells are in close apposition but tight junctions are rarely encountered and are rudimentary. The cytoplasm is poorly differentiated with sparse organelles. The majority of cells contain glycogen. Occasional perinuclear filaments may be found. The large-cell variant exhibits a greater number of cytoplasmic organelles, and well-developed desmosomes may be found with intermediate filaments.

The demonstration of intracytoplasmic glycogen is an aid in diagnosis. It is anticipated that all bona fide Ewing's sarcomas will demonstrate glycogen at some level of study. Light microscopic confirmation of the glycogen (PAS-positive and diastase-digestible) is very dependent on immediate fixation in 80% alcohol or at the least a transient exposure to 10% formalin. Imprints of the tumor with requisite staining are suggested. Kissane et al[6] indicate that 23% (71 of 368) of Ewing's sarcomas of bone yielded negative glycogen histochemical reactions, most often attributed to improper processing of tissue. The value of glycogen positivity is mitigated when it is appreciated that other important lesions in the differential diagnosis (neuroblastoma, embryonal rhabdomyosarcoma, osteosarcoma, mesenchymal chondrosarcoma, and even rare lymphomas) also may demonstrate glycogen to some degree.

Ewing's sarcoma is a biologically very aggressive malignancy. When managed along traditional lines of radical surgery or radiotherapy, the results are poor. This is underscored by the observation that of the more than 1,000 patients with Ewing's sarcoma reported in the world literature by 1975, only about 100 survived for 5 years or longer.[12] The high degree of lethality relates to the tumor's rapid growth (many have a doubling time of less than 25 days) and a considerable propensity for early dissemination.[13] As a result, there is a high probability of micrometastases before the tumor has even attained diagnosable size. This has stimulated trials with adjuvant systemic therapy designed to eradicate microscopic metastases. Data appear to support an increase in disease-free intervals and apparent cure rates with combined modality management. Actuarial projections of disease-free survival at 5 years of 33% to 75% have been recorded.[14] It is, however, clear that gross soft tissue extension is unfavorable, with respect to not only local failure but also distant metastases (lungs, skeleton, regional lymph nodes, CNS).[15]

Whether or not improvement of survival will accrue to Ewing's sarcoma of the jaws is not known. Arafat et al[5] indicate that only three of 18 patients with gnathic Ewing's sarcoma were alive 5 or more years after treatment.

The prognosis for extraskeletal Ewing's sarcoma is also evolving toward improvement in the era of combined modality therapy. Formerly, 75% of the patients died of metastases to lung or bone, often within 1 year of diagnosis. Now, a disease-free survival of 64% for 3 to 7 years after completion of treatment is possible.[7,16]

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OBITUARY

MICHELE ARSLAN, 1904-1988

Michele Arslan, MD, Professor Emeritus of Otolaryngology, University of Padua, Italy, died on Nov 22, 1988, at his home in Padua. He was 84 years old.

Dr Arslan was born in Padua on Jan 23, 1904, the son of an Armenian immigrant, who also was an otolaryngologist. He studied in Padua, taking his medical degree at the Padua University School of Medicine in 1927. He then went to Germany to continue his studies at Dr M. H. Fischer's Department of Physiology, where he learned the fundamentals of physiopathology of the vestibular system and perfected his knowledge of the German language. He entered the Department of Otolaryngology at the University of Padua, which was under the direction of his father, Yervant. In 1937, he was appointed Director of this Department on his father's retirement and held this position until 1974.

Dr Arslan contributed a good deal to our understanding of fundamental pathophysiology as well as clinical problems, particularly vestibular disorders. He made interesting contributions to the literature on the medical and surgical treatment of Meniere's disease, and also wrote several works on the history of medicine.

Dr Arslan's managerial abilities were made apparent when he was appointed President of the Xth World Congress of Otorhinolaryngology, held in Venice in 1973. He was President of the International Federation of Oto-Rhino-Laryngological Societies. In 1972, he became the first Italian corresponding member of the American Laryngological Association. He was a Fellow of the Royal Society of Medicine of London and of the Collegium Oto-Rhino-Laryngologicum Amicitiae Sacrum and was a Chevalier of the Legion of Honor. He was awarded honorary degrees by the universities of Uppsala and Strasbourg. He was a member of the Editorial Board of the *Annals of Otology, Rhinology & Laryngology*. He also received many national and international awards.

Apart from his involvement in medicine, Dr Arslan had a lively interest in the visual arts and classical literature. He was also a fervent Catholic. He had a particular interest in rugby and for 30 years was directly involved in developing the educational aspects of this sport in teams of young people.

Dr Arslan was not only a distinguished physician but also an excellent teacher and fine lecturer. His many pupils will remember him for his medical teaching and his charismatic personality.

He is survived by his wife, Vittoria, and five children: Antonia, Paola, Gianni, Carlo, and Edoardo, who is Professor of Audiology at the University of Ferrara.

Alfio Ferlito, MD

BOOK REVIEWS

The Human Vocal Tract: Anatomy, Function, Development, and Evolution

Edmund S. Crelin. Hard cover, illustrated, indexed, 265 pages, 1987. Vantage Press, New York, $25.

Dr Crelin's intent in writing this text was to enlighten the "uninitiated" reader and inspire students and investigators to conduct further research in the anatomy, function, development, and evolution of the human vocal tract. The author manages to effectively bind a broad range of topics in a text that is orderly, readable, and informative for the layperson and the professional. The book contains a bibliography and index, the latter perhaps too brief for the broad scope of the book's contents.

Throughout the text is a wealth of photographs and drawings that are helpful in comprehending the written descriptions. Of particular interest are the photographs of rubber vocal tract models (with the capacity to vocalize) constructed by the author to demonstrate vocal tract form and function. These models, made from casts of human and other animal bodies and fossils, play an integral role in the author's theory of the development and evolution of the human vocal tract.

Descriptions in the chapter on the anatomy and function of the human vocal tract are not as extensive as might be hoped for by the layperson, but are quite adequate for the reader who is familiar with the subject. Common names for some of the more difficult terms are presented parenthetically. One questionable passage describes esophageal speech as a process of "belching" air that has been "swallowed" into the esophagus. The laryngectomized patient who attempts to speak by "swallowing" air will soon become uncomfortable because of an air-filled stomach, and an accomplished esophageal speaker may be quite offended if told he sounds as if he is "belching."

The chapter concerning the development of the human vocal tract contains many intriguing topics, including comparative animal studies, obligate nose breathing, a theory of one possible cause of sudden infant death syndrome, speech defects, and developmental changes occurring in the vocal tract and skull during maturation. Pertinent to the author's theory of human evolution is the section describing chimpanzee vocal tract development. The author states that chimpanzees communicate but "don't have the capacity for true language." He then describes the functional limitations of the chimpanzee's vocal tract that prevent production of articulate speech. This is later related to the similar limitations of the earliest hominids and their theoretical incapacity for true language.

In the final chapter, Dr Crelin refers to the maturation of the human vocal tract as a "resume of the evolution of the tract." This statement reminds one of the theory that ontogeny recapitulates phylogeny and, considering the controversy surrounding this theory, must be read with caution. The author proceeds to describe a fascinating theory of human evolution based upon the changes in the vocal tracts and skulls of the earliest hominids through early modern *Homo sapiens* and on to present-day humans. He gives detailed descriptions of the research procedures that led him to his particular view of human evolution.

Finally, Dr Crelin attributes the ultimate appearance of human language and conceptual thought to the evolutionary progress in vocal communication followed by the development of articulate speech. He states, "This phylogenetic event is recapitulated in the development of every present-day human being between birth and six years of age." The author expresses his dismay at the destructive tendencies of the human race in view of its capacity for beneficial acts, the latter being the result of the "genetic mutations" that led to changes in the vocal tract and, ultimately, to language and thought.

Overall, this book contains interesting information for both the layperson and the professional, and does indeed inspire one to delve further into the research areas presented. This volume would be a valuable addition to the personal library of anyone interested in human and comparative anatomy and physiology; speech, language, and cognition; or human evolution.

Ferne McClintock, MS, CCC-SLP
Birmingham, Alabama

High-Resolution Computed Tomography of the Paranasal Sinuses, Pharynx and Related Regions — Impact of CT Identification on Diagnosis and Patient Management

Gertrude Maatman. Hard cover, illustrated, indexed, 171 pages, 1986. Martinus Nijhoff, Dordrecht, the Netherlands, $60.50.

This text, actually more of a combined text/atlas, is the most recent addition to a radiology series from Martinus Nijhoff Publishers. The strongest contribution comes in the first four chapters on introduction, technique, normal anatomy, and CT identification; these portions actually comprise half the book. The description of the technique is quite concise, reviewing axial, coronal, and even sagittal imaging methods. Using the supine position for obtaining direct coronal images in selected patients is not mentioned. From a dosimetry and radiation protection perspective, it is not mentioned that the slice angulation chosen from the lateral scanogram can be prescribed to specifically avoid lens exposure. As to the method of scanning axially at some angle to the orbitomeatal line, it is not clearly stated whether it is above or below that line.

In the anatomy and computed tomography identification chapters, the larynx has been intentionally excluded. The anatomic descriptions are concise though clearly detailed enough for the specialist. Smooth reading is compromised by the dryness of the text here and the strong leaning toward conventional Latin nomenclature. Some of the black-and-white line drawings are laboriously complex to review. The chapter on CT identification strongly exemplifies and supports the well-known tenet that while the normal anatomy has always existed, true progress has been made in the exquisite detail in which it can now be noninvasively imaged. The CT images, both axial and coronal, with and without intravenous contrast enhancement, are excellent. Though optimal for spatial resolution, one might reasonably question whether some pathologic anatomy might go undetected by the use of slice increments discrepantly much greater than individual slice thickness. Photography at soft tissue and bone window settings is demonstrated. An occasional contradiction between text and atlas is present, eg, the pharyngeal recess (fossa of Rosenmüller) is illustrated anterior to the torus tubarius but described in the text as being bounded anteriorly by the torus tubarius.

Two chapters constitute the majority of the second half of the book, one covering disorders of the paranasal sinuses, and the other, disorders of the pharynx and oral and nasal cavities. These chapters include specific cases and excellent images as well as compiled data to support the conclusion that CT has an indispensable role in influencing management of patients with pathologic processes in these anatomic regions. The spectrum of disease is not complete, however. For example, pediatric malignant tumors including neuroblastoma and rhabdomyosarcoma are not touched and congenital anomalies such as choanal atresia are not mentioned.

The index is moderately complete. Paper quality, readability of print, and photography are excellent. Scattered typographic errors are present but few in number.

In summary, this text is strongly competitive with others in the field from the standpoint of normal CT anatomy of the oronasal cavities, pharynx, and paranasal sinuses. Its few weaknesses should not deter purchase for the library of the ear, nose, and throat specialist or the radiologist who is confronted with evaluation of these areas.

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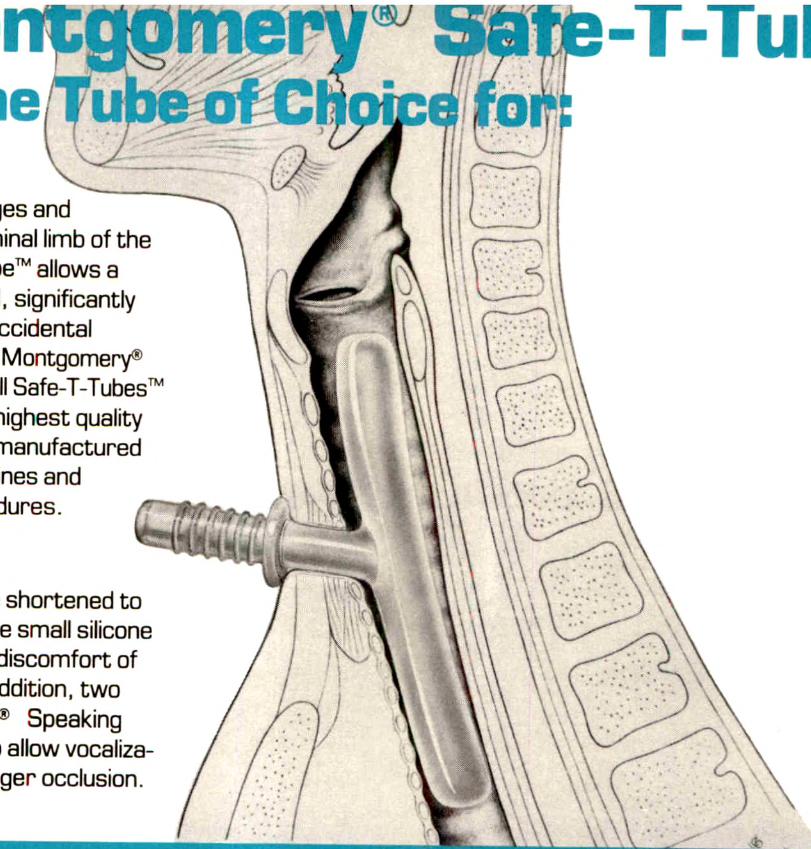

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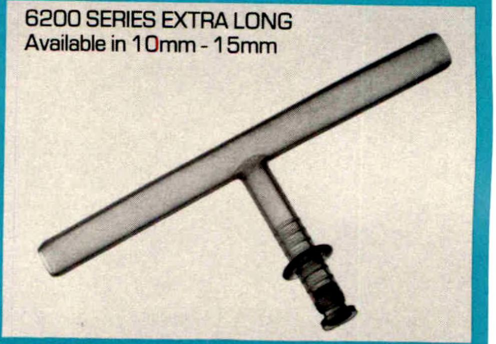

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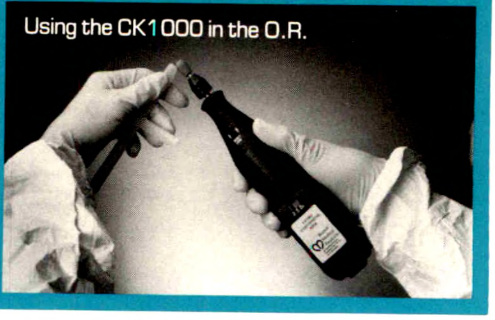

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GASTROESOPHAGEAL REFLUX AND POSTERIOR LARYNGITIS

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Esophageal acid exposure was assessed by 23-hour ambulatory pH monitoring and compared with a biopsy of the posterior larynx and proximal esophagus in 97 patients with hoarseness, burning pharyngeal discomfort, or globus sensation. Patient results were compared with normal acid exposure times obtained in 54 control subjects. In 24 patients there were laryngeal abnormalities but both esophageal biopsy results and acid exposure times were normal. Laryngeal disease was found in association with prolonged acid exposure time or esophagitis in only 17 of the 97 patients (17.5%) studied. Recent reports appear to have overestimated the importance of acid reflux as a cause of posterior laryngitis.

KEY WORDS — gastroesophageal reflux, posterior laryngitis.

INTRODUCTION

Esophagopharyngeal reflux first was suggested as an etiologic factor in laryngeal disease in 1968 by Cherry and Margulies,[1] who proposed laryngeal overspill of acid as a supplementary factor to vocal abuse[2] in the causation of contact ulcer of the larynx on the basis of cine-esophagraphic and acid barium studies in three patients. The theory was supported by the experimental production of laryngeal ulcers by the application of gastric acid to the vocal cords of two dogs.[3] Delahunty[4] later extended the spectrum of reflux-associated laryngeal disease to include keratosis of the posterior third of the larynx. Several other studies supported gastroesophageal reflux as an etiologic factor in laryngeal ulcer or granuloma, posterior laryngitis, or otherwise inexplicable hoarseness.[5-10] All of these relied on radiographic study or "clinical" evidence (history of heartburn or response to antacid regimen) to diagnose abnormal reflux.

Two more recent preliminary communications have implicated reflux in hoarseness[11] and posterior laryngitis[12] on the basis of prolonged ambulatory pH monitoring, which is the currently accepted best method for the diagnosis of abnormal esophageal acid exposure.[13] In neither study, however, were the results of pH monitoring compared with those of posterior laryngeal biopsy. The aim of the present study was to compare the results of prolonged monitoring of intraesophageal pH with those of posterior laryngeal biopsy in a large number of patients with a variety of laryngopharyngeal symptoms.

METHODS

From October 1986 to August 1987, 97 patients (30 men, 67 women) 22 to 86 years of age (mean, 50.3 years) were studied. A detailed laryngeal history was taken in the voice clinic together with videorecording and stroboscopic inspection of the larynx.

Direct inquiry about gastrointestinal symptoms was made and heartburn was scored as 0 = no heartburn; 1 = less than once per month; 2 = less than once per week; and 3 = more than once per week. Patients were selected for the study on the basis of the "classic" features of "acid laryngitis," ie, grade 2 or 3 heartburn in association with vocal symptoms and/or globus sensation; burning pharyngeal discomfort; nocturnal choking or evidence of posterior laryngeal inflammation or pachyderma at laryngoscopy. Ten patients had a principal complaint of globus sensation without heartburn or marked vocal symptoms.

The pH monitoring control group comprised 1) 34 asymptomatic volunteers (27 men, seven women) 19 to 67 years of age (mean, 34 years) and 2) 20 patients (seven men, 13 women) 31 to 69 years of age (mean, 52 years) with noncardiac chest pain whose radiographic, manometric, and endoscopic investigations yielded normal results.

Prolonged ambulatory pH monitoring was performed in 150 subjects. One elderly man with histologically confirmed esophagitis declined the investigation. A Radiometer (VA Howe, London) glass/KCl electrode with combined reference electrode was sited 3 cm above the proximal end of the lower esophageal sphincter as determined by prior station pull-through. This was linked to a digital pH meter (Synectics) strapped to the subject's waist. Computer analysis of the stored data was used to derive the percentage of time the intraesophageal pH was below 4 (total and with patients in erect and recumbent positions), total number of reflux

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TABLE 1. CLINICAL FEATURES

Group	*n*	*M*	*F*	*Age (yr) Mean ± SD*	*Heartburn More Than Once/Month*	*Hoarseness*	*Globus Sensation*
Reflux, no laryngitis	14	4	10	49.3 ± 14.3	10	7	11
Reflux, laryngitis	17	9	8	54.8 ± 12.9	13	13	9
Laryngitis, no reflux	24	3	21	47.0 ± 14.3	7	13	20
No laryngitis, no reflux	42	14	28	49.6 ± 11.3	14	20	39
Total	97	30	67	50.3 ± 13.3	44	53	79
Control subjects	54	34	20	40.5 ± 15.5	2	—	—

episodes ($\mathrm{pH}<4$), longest reflux episode, and number of episodes greater than 5 minutes.

Patients underwent barium meal examination followed by direct laryngoscopy and rigid esophagoscopy under general anesthesia. Biopsy samples were taken from any structural abnormality, the interarytenoid area of the larynx, and the proximal esophagus. All specimens were examined by a single observer (J.P.), who was not informed of the results of other investigations and who noted any epithelial or inflammatory changes.

Results were analyzed with Student's t test on log-transformed data, the χ^2 test with Yates' correction, and the Wilcoxon rank sum test. As pH monitoring data in control subjects is not normally distributed but shows a positive skew, it is not possible to define a normal range based on arithmetic mean and standard deviation. Although it has been suggested recently that upright and recumbent acid exposure times be used separately to distinguish normal from pathologic reflux,[14] we relied on total percentage time $\mathrm{pH}<4$ in the present study, as the literature is unclear on the relative importance of diurnal and nocturnal reflux in the causation of laryngeal symptoms. Any patient, then, whose total time $\mathrm{pH}<4$ was greater than 10% or whose proximal esophageal biopsy results showed evidence of acute or chronic inflammatory infiltrate was classed as a "reflux patient."

RESULTS

Patients were classified into four groups—reflux, no laryngitis; reflux, laryngitis; laryngitis, no reflux; no laryngitis, no reflux—and the clinical features are summarized in Table 1. Mean age was similar in the four groups. Greater than monthly (grade 2 to 3) heartburn was much commoner (82%) in the two reflux groups than in the two nonreflux groups (32%) ($\chi^2 = 13.6$, $p<.001$). The ten patients with isolated globus sensation were among the 42 with neither laryngitis nor reflux. Burning sore throat was an uncommon symptom and was present in only four patients, all with laryngitis, three with reflux. Around one third of patients were cigarette smokers (Table 2). There was an increased incidence of smoking in laryngitis patients without reflux ($p = .05$ compared with all nonlaryngitis patients; $p<.05$ compared with nonlaryngitis, nonreflux patients).

TABLE 2. SMOKING

Group	*n*	*Smokers*
Reflux, no laryngitis	14	6
Reflux, laryngitis	17	5
Laryngitis, no reflux	24	13*
No laryngitis, no reflux	42	9
Total	97	33

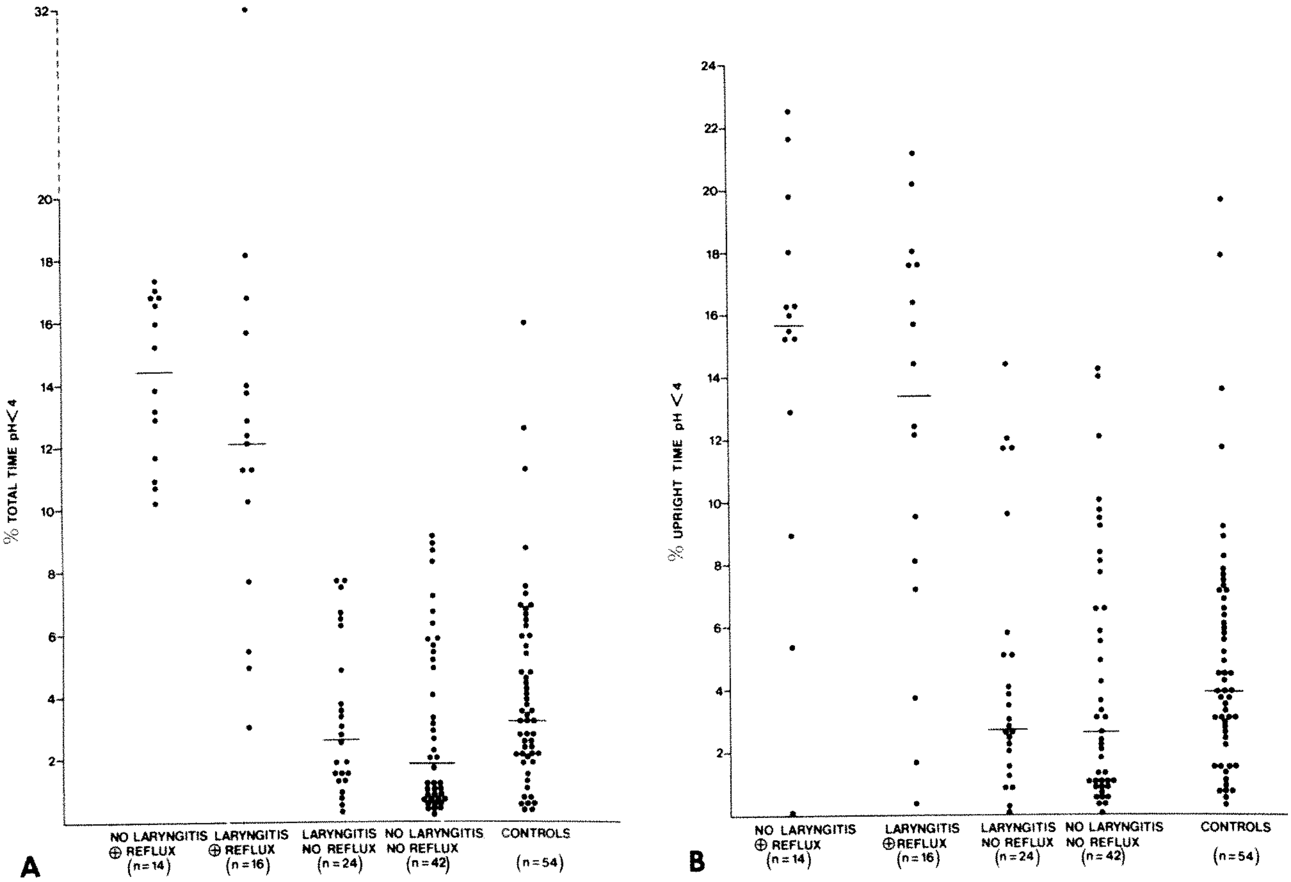
*$\chi^2 = 3.84$, $p = .05$ compared with patients without laryngitis.

Results of pH monitoring are summarized in Figs 1 and 2. Total and upright acid exposure times (Table 3) were log transformed prior to analysis to provide a normal distribution. Patients in the two reflux groups had significantly greater total acid exposure time ($p<.001$, Student's t test) and also, to a lesser extent, greater upright percentage time $\mathrm{pH}<4$ ($p<.01$). The large number of patients (Fig 1C) with no supine reflux precludes normalization by log transformation. Accordingly, data were analyzed by the Wilcoxon rank sum test. Again, the reflux groups both had significantly greater acid exposure ($p<.01$, Table 4). The total number of reflux episodes (Fig 2) was not a valuable discriminating factor but the number of episodes greater than 5 minutes (Table 5) confirmed the prolonged acid exposure in all the reflux patients. There was no significant difference between reflux patients with and without laryngitis in total, upright, or recumbent percentage time $\mathrm{pH}<4$, nor in the number of episodes greater than 5 minutes.

Videolaryngoscopy was a reliable predictor of histologic abnormality in 90% of patients. The commonest pathologic finding in the 41 patients with posterior laryngeal abnormality was of chronic inflammatory infiltrate in 27 patients (Table 6). This was usually a mixed cellular infiltrate, but in a few patients lymphoid cells predominated and occasionally mast cells were prominent. Parakeratosis was noted in 15 patients, usually in association with another abnormality. Four patients (three with reflux, two smokers) had vocal cord abnormalities (nodules or inflammation). No pathologic feature appeared to be characteristic of patients with reflux.

DISCUSSION

In recent years an increasing number of otolaryngic manifestations of gastroesophageal reflux have been reported including not only posterior



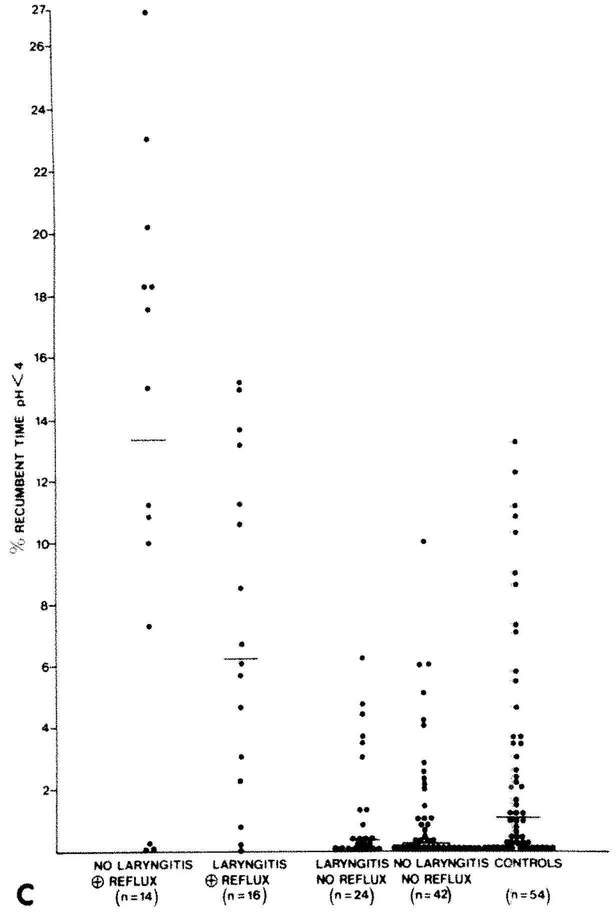


Fig 1. Esophageal acid exposure. Median is indicated. A) Total. B) Upright. C) Recumbent. One subject with laryngitis and reflux not shown (percentage of time recumbent was 49%).

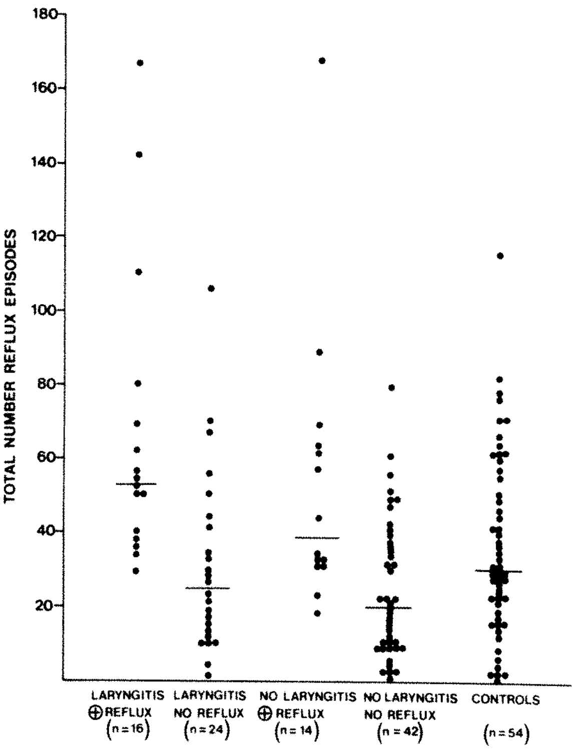


Fig 2. Total number of reflux episodes. Median is indicated.

laryngitis but also globus sensation, cricopharyngeal hypertrophy, upper esophageal webs, pharyngeal pouch, otitis media, and subglottic stenosis.[15,16] We have shown previously that abnormal esophageal acid exposure is present in just 15% of globus patients.[17] The results of the present study indicate that reflux is also an uncommon factor in posterior laryngitis. In a study of 14 patients with chronic hoarseness, Wiener et al[11] showed some abnormality of acid exposure times in almost 80% of patients. Selection criteria were not cited, however, and the wide variety of laryngeal disorders (inflammation, granuloma, stenosis, carcinoma, and previous tracheostomy), the small number of subjects studied, and the possibility of sphincteric incompetence and laryngeal overspill in some of these suggested to us that the importance of reflux may have been overestimated.

It also has been claimed recently that patients with abnormal esophageal acid exposure show posterior hyperkeratotic white plaques detectable at transnasal fiberoptic laryngoscopy.[12] These were noted in 92% of patients with reflux and in 78% of patients with reflux and asthma, but in only three patients were biopsies performed on the plaques. Although an incidence of gastroesophageal reflux of 20% to 65% in asthmatics has been reported for many years,[18-20] there is no other report of such laryngeal lesions, and the possibility of an association with inhaler therapy, in particular steroid sprays, cannot be excluded, nor the possibility of misinterpretation of laryngoscopic findings. Reflex bronchoconstriction may result from aspiration of even microscopic amounts of gastric content or by stimulation of acid-sensitive esophageal receptors. Conversely, asthma may induce reflux by alterations in diaphragmatic function, and theophylline therapy may reduce lower esophageal sphincter pressure. Pellegrini et al[21] were able to confirm aspiration by pH monitoring, ie, pH drop followed by acid taste and cough or wheeze, in only eight of 48 patients with clinically suspected aspiration. More recently, combined hypopharyngeal and proximal esophageal pH monitoring has been reported by Wiener et al[22] in a heterogeneous group of patients with hoarseness, laryngeal tumors or respiratory symptoms, and abnormal gastroesophageal reflux. Again, gastroesophagopharyngeal reflux, long thought to be the mechanism by which reflux might produce laryngeal disease,[1,7,8] was found to be extremely rare.

The response of proximal esophageal epithelium to physiologic and pathologic degrees of acid reflux is well documented.[23,24] The histologic response of upper respiratory tract epithelium to acid has been studied only in experimental animal studies[3,16,25] that bear little relationship to laryngeal acid exposure in vivo. In the present study, 41 patients with posterior laryngeal abnormality have been identified (Table 6) but only 17 (41%) had evidence of gastroesophageal reflux. A similar number of patients (18) were smokers and the incidence of smok-

TABLE 3. GEOMETRIC MEAN TIME pH<4

Group	*Total (%)*	*Upright (%)*
Reflux, no laryngitis	13.98*	10.35†
Reflux, laryngitis	10.76*	7.20†
Laryngitis, no reflux	2.21	2.48
No laryngitis, no reflux	1.87	2.42
Control subjects	2.97	3.57

*p<.001, Student's *t* test.
†p<.01, Student's *t* test.

TABLE 4. RECUMBENT TIME pH<4

Group	*Median (%)*	*Range (%)*
Reflux, no laryngitis	13.2*	0-27.2
Reflux, laryngitis	7.6*	0-49.0
Laryngitis, no reflux	0.3	0- 6.2
No laryngitis, no reflux	0.2	0-10.0
Control subjects	1.0	0-15.4

*p<.01, Wilcoxon rank sum test.

TABLE 5. NUMBER OF REFLUX EPISODES GREATER THAN 5 MINUTES

	No. of Episodes >5 min			
Group	*0-3*	*4-6*	*7-9*	*>9*
Reflux, no laryngitis	1	1	8	2
Reflux, laryngitis	1	5	6	4
Laryngitis, no reflux	21	1	2	—
No laryngitis, no reflux	34	6	—	1
Control subjects	42	9	1	2

Data are numbers of patients.

TABLE 6. LARYNGEAL DISORDERS (n = 41)

	No. of Patients	*Patients With Reflux (n = 17)*	*Smokers (n = 18)*
Interarytenoid			
Chronic inflammation	27	10	13
Parakeratosis	15	7	7
Submucosal scarring	2	2	—
Edema	2	1	—
Vocal cord			
Nodule	2	1	2
Inflammation	2	2	—
Postcricoid web	2	—	1

ing was lower in those with both reflux and laryngitis than in those with laryngitis alone (although not significantly), a finding previously reported by Öhman et al[26] and others (J. E. Richter, personal communication). Results of pH monitoring are, however, not greatly influenced by cigarette smoking.[27] Since 11 patients with laryngitis were neither refluxers nor smokers, other factors such as voice abuse may be relevant to posterior laryngitis. This should be borne in mind in assessing uncontrolled studies in which reflux is assessed "clinically," as heartburn is present in around 30% of the general middle-aged population (and in the nonreflux patients in the present study; Table 1). Unlike Wiener et al,[11] we found heartburn to be equally common in reflux patients with and without laryngitis (Table 1).

Of 31 reflux patients, 20 complained of hoarseness (64%; Table 1) and 17 had posterior laryngeal changes (55%). Three patients with hoarseness and abnormal esophageal acid exposure had no pathologic lesion to account for their dysphonia. While aspiration of very small amounts of acid might produce symptoms in the absence of histologic abnormality, this may again simply reflect a chance coincidence of two common symptoms in the patients reported, whose mean age (50 years) is similar to that of other series. Similarly, 20 of the 42 patients with neither laryngitis nor reflux had hoarseness. Globus sensation was the predominant symptom in ten patients and we believe that many of these patients have a functional basis for their symptoms.

Over the 10-month study period, approximately 250 patients attended our dysphonia clinic but none had evidence of contact ulceration and even substantial series of contact ulcers report no more than six or seven cases per annum.[8,26] There are no reports of pH monitoring in this variant of posterior laryngitis, although the importance of underlying stress and of speech therapy has been recognized even by those claiming a high incidence of esophageal dysfunction in the condition.[26]

The 17 patients with evidence of "acid" laryngitis represent an overall incidence in our dysphonic clinic patients of around 7%, although this figure is, of course, speculative. Unfortunately, the investigation of every attender at the voice clinic by pH monitoring and interarytenoid biopsy under general anesthesia is logistically and ethically unacceptable.

We do not confirm an abnormal degree of upright reflux (Table 3) nor a high incidence of normal findings in the proximal esophagus in patients with reflux and laryngitis.[11] It has been shown that esophagoscopy is complementary to pH monitoring in the diagnosis of gastroesophageal reflux[28] and only three of 17 patients with reflux-associated laryngeal disease had normal proximal esophageal biopsy results. Normal endoscopic findings in 72% of their patients with hoarseness and apparently abnormal pH monitoring data lead us to suspect that the high incidence (61%) of abnormal total percentage time pH<4 in the series of Wiener et al[11] may result not just from differences in patient selection but also from inappropriate use of parametric statistical methods.

CONCLUSIONS

We have found evidence of concurrent gastroesophageal reflux and posterior laryngitis in 17 of 97 patients studied (17.5%). Fourteen patients had reflux without laryngitis and 24 had laryngitis without reflux. Posterior laryngeal changes are similar in patients with and without documented reflux. Cigarette smoking and psychologic factors appear to be additional etiologic features in some patients. We believe that our figure of 17.5% more accurately reflects the true incidence of "acid laryngitis" than studies based on clinical impressions and uncontrolled trials of medical therapies, some of which involve cessation of smoking and none of whose placebo component has been assessed. Even so, this is probably a considerable overestimate as all but ten of the patients were selected as likely candidates for the condition. A more accurate measurement of the incidence in unselected dysphonic patients is hard to establish because of the complexity of the methods used.

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THE INTERNATIONAL SYMPOSIUM ON CLINICAL AND BASIC OTOLOGY

The International Symposium on Clinical and Basic Otology will be held June 4-6, 1990, in Beijing, People's Republic of China. The abstract deadline is December 31, 1989. For further information, contact the Beijing Institute of Otorhinolaryngology, Chan Liu, MD, Professor and Director, 17 Hougou Lane, Beijing 1000005, People's Republic of China.

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SURVIVAL OF SPIRAL GANGLION CELLS IN PROFOUND SENSORINEURAL HEARING LOSS: IMPLICATIONS FOR COCHLEAR IMPLANTATION

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Ninety-three temporal bones from 66 patients who were profoundly deaf during life were reconstructed by analysis of serial light microscopic sections. The correlations of total and segmental spiral ganglion cell counts with age, duration of hearing loss and profound deafness, and cause of hearing loss were evaluated. Bivariate analysis demonstrated that total spiral ganglion cell count tended to be lower in older than in younger deaf individuals and lower with longer duration of hearing loss and total deafness. However, multiple regression analysis demonstrated that the cause of hearing loss was the single most significant determinant of total spiral ganglion cell count. Patients with deafness due to aminoglycoside toxicity or sudden idiopathic deafness had the highest residual spiral ganglion cell count and patients with deafness due to presumptive postnatal viral labyrinthitis, bacterial labyrinthitis, and congenital or genetic causes had the lowest numbers of residual spiral ganglion cells.

KEY WORDS — cochlear implantation, profound deafness, sensorineural hearing loss, spiral ganglion cells.

INTRODUCTION

The introduction of electrical stimulation of the inner ear and auditory nerve as a means of treating profound sensorineural hearing loss has added new significance to the histopathology of the various causes of deafness.

Although the degree of preservation of several elements of the inner ear in deaf patients may have relevance to the potential success of cochlear implantation, the preservation of the first order cochlear neuron and its cell body in the spiral ganglion is generally considered to be of primary importance.[1-5]

To date there have been few quantitative studies of the preservation of the spiral ganglion in the deaf human inner ear.[2-8] The present study is an analysis of 93 profoundly deaf ears in 66 patients. The total spiral ganglion counts were correlated with diagnostic category, age at death, age at onset of hearing loss, duration of hearing loss, age at onset of total deafness, and duration of total deafness to evaluate the most significant predictors of total spiral ganglion cell count in deaf ears.

MATERIALS AND METHODS

In the human temporal bone collection at the Massachusetts Eye and Ear Infirmary, there were 93 temporal bones from 66 patients who during life had a documented profound sensorineural hearing loss. Twelve diagnostic categories and the numbers of temporal bones in each category are presented in Table 1.

All temporal bones were fixed in Heidenhain Susa solution, decalcified in trichloroacetic acid, and embedded in celloidin.[9] The temporal bones were sectioned at a thickness of 20 μm in the horizontal plane, that is, nearly parallel to the middle fossa floor. Every tenth section was stained with hematoxylin and eosin and mounted on glass slides. The

TABLE 1. CAUSES OF PROFOUND SENSORINEURAL HEARING LOSS

Category	*Diagnosis*	*No. of Patients*	*Mean Age at Death (yr)*	*No. of Temporal Bones*
1	Postnatal viral labyrinthitis	8	53	13
2	Congenital rubella syndrome	2	12	3
3	Sudden idiopathic deafness	6	56	6
4	Congenital syphilis	2	70	3
5	Bacterial labyrinthitis	11	71	15
6	Tumor of temporal bone or cerebellopontine angle	8	45	10
7	Otosclerosis and/or presbycusis	3	63	5
8	Congenital or genetic cause	9	40	15
9	Trauma or fracture of temporal bone	4	57	4
10	Aminoglycoside ototoxicity	8	41	13
11	Meniere's disease	2	74	2
12	Unknown cause	3	80	4
Total		66		93

Also studied were five temporal bones from five normal-hearing subjects whose mean age at death was 39 years.

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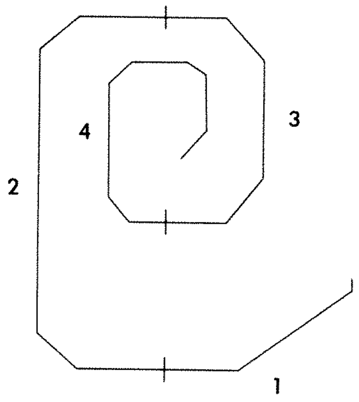

Fig 1. Diagrammatic representation of technique for reconstruction of human spiral ganglion by serial section analysis.

organ of Corti and spiral ganglion were graphically reconstructed by a method described by Schuknecht[10,11] and Otte et al[5] using an ocular grid. All ganglion cells with visible nucleoli were counted on every tenth section. Counts were multiplied by 10 to account for unmounted sections, and also by a factor of 0.9 to account for doubly counted spiral ganglion cells.[11] The ganglion cell populations were determined for each of four segments of the spiral ganglion (Fig 1). Counts of spiral ganglion cells in each of the four segments were added to achieve a total spiral ganglion cell count. Similarly, in each section examined the presence or absence of inner and outer hair cells and pillar cells was recorded.

For statistical analysis, a mean total spiral ganglion cell count and mean segmental counts were computed for each of the 12 diagnostic categories. In addition, the total spiral ganglion cell count was correlated with the age of the patient at death, the age at onset of hearing loss, the age at onset of total deafness, the duration of hearing loss (age at death minus age at onset of hearing loss), and the duration of deafness (age at death minus the age at onset of total deafness). The data from left and right ears were analyzed separately because a number of individuals contributed data from two ears. Segregation of data from left and right ears allowed adjusting for the statistical correlation between left and right ears from the same individual and a more accurate estimate of standard error and determination of p values.

In addition, the analysis was repeated by choosing one ear at random from individuals with measures of spiral ganglion cell count in both ears and using a table of 10,000 random digits. Differences between diagnostic groups in mean total spiral ganglion cell count were examined initially by analysis of variance for the six most common diagnostic groups, followed by *t* tests to examine differences between pairs of diagnostic groups. In addition to presenting nominal p values from these *t* tests, the Bonferroni approach[12] was used to assess statistical significance by adjusting for multiple comparisons. These analyses were repeated to assess differences between diagnostic groups in mean spiral ganglion cell count for each of the four segments. A cross-classification table, with associated χ^2 statistic, was also used to describe differences among diagnostic groups in total spiral ganglion cell counts. Because the distributions of percent remaining hair cells and pillar cells were highly nonnormal, associations of these variables with diagnostic groups were evaluated solely by cross-classification tables. Pearson correlation coefficients were used to examine the strength of associations between spiral ganglion cell counts in each of the four segments and in total counts, as well as the association of total spiral ganglion cell counts with age at death, age at onset of hearing loss, duration of hearing loss, age at onset of deafness, and duration of deafness. Multiple linear regression was used to evaluate the association of these age and duration measures with total spiral ganglion cell count adjusted for the effects of diagnostic groups.[13] All statistical analyses were performed with BMDP[14] statistical software.

RESULTS

The results of the analyses using right and left ears separately and choosing one ear at random from patients in whom two ears were measured were very similar. Since the correlation between spiral ganglion cell counts in pairs of ears of the 27 individuals with two measures was 0.87, and because the analysis using an ear at random increased the numbers in each diagnostic category and hence the power of analysis, only the results using one ear at random are presented.

The diagnostic categories of cause of profound sensorineural hearing loss of the 93 temporal bones from 66 patients are shown in Table 1. The means and standard deviations of total and segmental spiral ganglion cell counts within diagnostic groups and from five individuals with normal hearing at death are presented in Table 2. The significant p values from analyses of variance indicate that mean total spiral ganglion cell counts are not equal across the six most common diagnostic groups (diagnostic categories 1, 3, 5, 6, 8, and 10). Segmental counts from segments 2 (corresponding to 6 to 15 mm from the basal end to the cochlea) and 3 (15 to 22 mm) were most closely correlated with total ganglion cell count. Differences in ganglion cell counts in segment 1 (0 to 6 mm) showed the weakest correlation with differences in total ganglion cell counts (Table 3).

The p values from separate variance *t* tests of pairs of diagnostic groups (Table 4) indicate that diagnostic group 1 differs significantly from diagnostic group 10 and possibly from diagnostic groups 3 and 6, that group 5 differs from group 10, and

TABLE 2. SEGMENT-SPECIFIC SPIRAL GANGLION CELL COUNTS WITHIN DIAGNOSTIC GROUPS (ONE EAR CHOSEN FROM EACH PATIENT)

Group	n	Segment 1 (Mean ± SD)	Segment 2 (Mean ± SD)	Segment 3 (Mean ± SD)	Segment 4 (Mean ± SD)	Total (Mean ± SD)
Diagnostic						
1	8	1,281 ± 1,062	2,649 ± 2,394	1,865 ± 1,170	2,084 ± 1,677	7,880 ± 5,760
2	2	2,113 ± 86	5,558 ± 83	2,876 ± 95	2,581 ± 162	13,127 ± 261
3	6	2,403 ± 1,289	8,765 ± 5,442	5,438 ± 2,935	5,238 ± 2,602	21,844 ± 11,637
4	2	684 ± 815	1,949 ± 1,190	1,503 ± 255	1,598 ± 655	5,733 ± 2,915
5	11	1,361 ± 786	4,471 ± 1,955	3,126 ± 1,143	3,009 ± 1,623	11,968 ± 4,367
6	8	2,149 ± 1,431	6,564 ± 3,526	4,529 ± 1,923	4,379 ± 1,743	17,620 ± 8,385
7	3	1,905 ± 1,156	7,320 ± 2,003	5,031 ± 981	4,629 ± 920	18,885 ± 4,612
8	9	1,557 ± 971	4,968 ± 3,028	2,597 ± 1,951	2,075 ± 1,901	11,197 ± 6,823
9	4	1,571 ± 1,488	4,331 ± 3,512	2,747 ± 2,398	2,819 ± 2,606	11,468 ± 9,152
10	8	2,522 ± 1,728	8,417 ± 2,117	5,837 ± 1,319	4,852 ± 1,115	21,628 ± 5,113
11	2	1,467 ± 344	4,797 ± 1,247	3,267 ± 904	3,195 ± 1,273	12,726 ± 3,080
12	3	915 ± 255	2,972 ± 858	2,004 ± 1,802	1,374 ± 1,364	7,265 ± 4,268
Total	66	1,732 ± 1,194	5,460 ± 3,394	3,570 ± 2,113	3,299 ± 2,031	14,061 ± 8,063
Normal hearing	5	3,105 ± 700	11,214 ± 1,421	7,186 ± 1,288	6,914 ± 1,668	28,418 ± 3,675

Analysis of Variance Between Groups 1, 3, 5, 6, 8, and 10

Segment 1	Segment 2	Segment 3	Segment 4	Total
F = 1.63	F = 4.57	F = 6.65	F = 4.81	F = 5.42
p = .17	p = .002	p = .0001	p = .001	p = .0006

See text for definition of segments.

that group 8 differs from group 10. If one corrects the p values by the more conservative Bonferroni approach because of multiple comparisons, only diagnostic categories 1 and 10, 5 and 10, and 8 and 10 have significantly different mean spiral ganglion cell counts. Analysis by segmental ganglion cell counts leads to the same conclusions.

By using the χ^2 test and a categorical approach to counts of remaining spiral ganglion cells, the null hypothesis that there was no association between spiral ganglion cell counts and the six most common diagnostic categories was rejected (Table 5). Most temporal bones from diagnostic group 1 demonstrated total counts below 10,000, whereas the majority of temporal bones from diagnostic groups 3 and 10 demonstrated counts above 20,000.

Table 5 also displays a categorical approach to the examination of the distribution of percent remaining hair cells and pillar cells within a diagnostic group. The highly significant χ^2 values indicate that the distribution of percent remaining hair cells and pillar cells differs across diagnostic groups. Thus, most ears from diagnostic groups 1 and 5 have no remaining hair cells or pillar cells, whereas most ears from diagnostic groups 6 and 10 have some or all pillar and hair cells remaining. In particular, all eight ears from diagnostic category 6 have a full complement of pillar cells.

Bivariate analysis of the correlation of total ganglion cell counts with a number of clinical variables is displayed in Table 6. This bivariate analysis demonstrated that 1) spiral ganglion cell count was lower in older than in younger deaf individuals, and 2) the total spiral ganglion cell count tended to be lower with longer duration of hearing loss and of total deafness. The strength of these correlations ranged from very weak to moderately strong. The only correlations that reached statistical significance were between the total spiral ganglion cell counts and the duration of hearing loss and duration of total deafness.

Because many of these correlates of spiral ganglion cell count were themselves intercorrelated, and because the underlying cause of deafness may be a confounding variable in these relationships, multiple regression analyses were performed with data from the 49 ears in the six most common diagnostic categories that demonstrated no missing values on any predictive variable. Regression co-

TABLE 3. CORRELATIONS BETWEEN SPIRAL GANGLION CELL COUNTS IN SPECIFIC SEGMENTS AND IN TOTAL (ONE EAR CHOSEN FROM EACH OF 66 PATIENTS)

	Segment 2	Segment 3	Segment 4	Total Count
Segment 1	.776	.627	.627	.797
Segment 2		.886	.793	.968
Segment 3			.853	.943
Segment 4				.902

TABLE 4. P VALUES FOR SEPARATE VARIANCE T TESTS OF PAIRS OF DIAGNOSTIC GROUPS FOR TOTAL SPIRAL GANGLION CELL COUNT (ONE EAR CHOSEN FROM EACH PATIENT)

	Group 3	Group 5	Group 6	Group 8	Group 10
Group 1	.031	.117	.019	.294	.0002*
Group 3		.094	.471	.081	.967
Group 5			.113	.774	.0007†
Group 6				.108	.272
Group 8					.0028†

*p<.01 by Bonferroni post hoc test.
†p<.05.

TABLE 5. DISTRIBUTION OF EARS BY CATEGORIES OF SPIRAL GANGLION CELL COUNT, REMAINING PILLAR CELLS, AND HAIR CELLS (ONE EAR CHOSEN FROM EACH PATIENT)

Diagnostic Group	*Spiral Ganglion Cell Count*			*Remaining Hair Cells*			*Remaining Pillar Cells*		
	<10,000	*10,000-20,000*	*>20,000*	*None*	*Some*	*All*	*None*	*Some*	*All*
1	7	1	0	5	3	0	5	2	1
2*	0	2	0	1	0	0	1	0	0
3	1	0	5	1	4	1	1	4	1
4	2	0	0	2	0	0	2	0	0
5	4	7	0	8	2	1	8	2	1
6	1	5	2	1	1	6	0	0	8
7	0	2	1	1	2	0	1	2	0
8	3	5	1	5	3	1	5	2	2
9	2	1	1	0	4	0	0	2	2
10	0	4	4	2	6	0	1	2	5
11	0	2	0	0	2	0	0	1	1
12	2	1	0	2	0	1	2	0	1
Total	22	30	14	28	27	10	26	17	22
		$\chi^2 = 34.3$			$\chi^2 = 31.2$			$\chi^2 = 31.9$	
		$p = .0002$			$p = .0005$			$p = .0004$	

χ^2 is for groups 1, 3, 5, 6, 8, and 10.

*In one ear with spiral ganglion count measured, it was not possible to measure percent remaining hair cells and pillar cells.

efficients are displayed in Table 7. The age at onset of hearing loss was not entered into the regression because it is highly correlated with the age at onset of deafness. Duration variables were not entered because they are linear combinations of onset and age at death. Controlling for the variables in the regression, a partial correlation of age at onset of hearing loss with spiral ganglion cell count was negligible (less than 0.10). These regression equations accounted for 45% of the variability of spiral ganglion cell counts.

Furthermore, consideration of the residuals from the predicted regression values revealed one notable outlier: an individual in diagnostic group 3 with no remaining spiral ganglion cells yet a predicted count of over 20,000. Because such an outlier can markedly influence a fitted regression equation, because this individual was otherwise remarkable since she had a full complement of hair cells and pillar cells remaining at death, and because of her early age at onset of deafness due to diagnosis 3, the regression equation was refitted excluding her data. This model based on data from 48 ears provided a much better fit. The R^2 for the ten-variable model was .670; for the model containing only the five indicators of diagnostic group the R^2 was .566; thus, eliminating the one outlier, the diagnostic group accounted for 57% of the variability in spiral ganglion cell counts. That is, the major determinant of spiral ganglion cell count at death was the diagnostic category rather than the age at death, duration of deafness, or duration of hearing loss.

TABLE 6. PEARSON CORRELATION COEFFICIENTS WITH SPIRAL GANGLION CELL COUNT (ONE EAR FROM EACH INDIVIDUAL)

Variable	*Correlation*
Sex	−0.10
Age at death	−0.24
Age at onset of hearing loss	0.22
Duration of hearing loss	−0.41*
Age at onset of deafness	0.18
Duration of deafness	−0.40†

*p<.001.

†p<.01.

Variables statistically significant at $p<.05$ in this ten-variable model were identical to those significant at this level in the comparable model including the outlier. The associations of spiral ganglion cell count with percent remaining pillar cells and later age at onset of deafness approached statistical significance (each p between .05 and .10) in this reduced data set.

DISCUSSION

Bivariate analysis demonstrated that the spiral

TABLE 7. SUMMARY OF MULTIPLE LINEAR REGRESSION ANALYSIS PREDICTING TOTAL SPIRAL GANGLION CELL COUNT FOR 49 EARS FROM DIAGNOSTIC GROUPS 1, 3, 5, 6, 8, AND 10 (ONE EAR CHOSEN FROM EACH PATIENT)

Variable	*Coefficient*	*Standard Error*
Diagnostic group 3	14,795*	4,452
Diagnostic group 5	5,293	3,458
Diagnostic group 6	11,029†	4,934
Diagnostic group 8	2,871	3,510
Diagnostic group 10	12,524*	4,271
Age at death	−36.2	56.5
Age at onset of deafness	−14.8	55.9
Sex	400	2,259
Constant	9,283	
	$R^2 = .446$	

One ear was deleted because age at onset of deafness was unknown. Coefficients for diagnostic group estimate mean difference in counts between ears from that diagnostic group and ears from diagnostic group 1. Coefficient for sex estimates mean difference in counts between ears of men and women. R^2 for model containing only five indicators of diagnostic groups = .425.

*p<.01.

†p<.05.

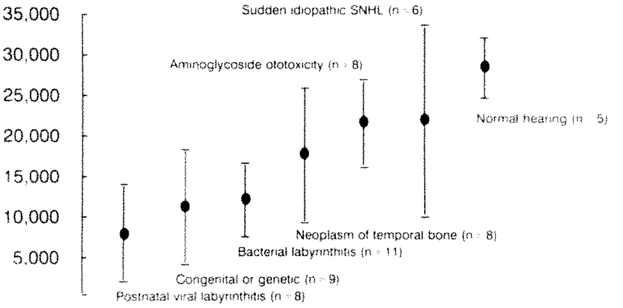

Fig 2. Means and standard deviations of spiral ganglion cell counts in six most common diagnostic categories and five individuals with normal hearing.

ganglion cell counts were lowest in temporal bones from individuals who were old at the time of death and individuals who had a long duration of hearing loss or a long duration of total deafness. The use of multiple regression analysis, however, suggested that the contribution of these variables was minimal and that the main determinant of the total spiral ganglion cell count was the cause of deafness. All analyses showed differences among the diagnostic categories of cause of deafness. Of the six most common diagnostic groups (groups 1, 3, 5, 6, 8, 10), temporal bones from diagnostic group 1 (postnatal viral labyrinthitis) have the lowest spiral ganglion cell counts, and there was evidence that the mean spiral ganglion cell count for diagnostic group 1 was significantly lower than the mean counts for temporal bones from diagnostic categories 3, 6, and 10. The mean spiral ganglion cell count for diagnostic group 10 (aminoglycoside ototoxicity) was significantly higher than the mean spiral ganglion cell count for temporal bones from diagnostic groups 1, 5, and 8. The means and standard deviations of spiral ganglion cell counts in diagnostic categories 1, 3, 5, 6, 8, and 10 and five individuals with normal hearing at death are presented in Fig 2.

There were also group differences in percent remaining hair cells and pillar cells. Temporal bones from diagnostic groups 1 and 5 were least likely to have hair cells and pillar cells remaining, whereas ears from diagnostic groups 6 and 10 were most likely to have all or some of such cells remaining.

Comparison of segmental analysis with total spiral ganglion cell counts suggested that segments 2 (corresponding to 6 to 15 mm from the basal end to the cochlea) and 3 (15 to 22 mm) were the most closely correlated and segment 1 (0 to 6 mm) the most poorly correlated with total counts. The poor correlation of counts in segment 1 with total counts in the various diagnostic categories is probably explained by the fact that loss of ganglion cells with advancing age is greatest in the basal turn.[15] In addition, horizontal section of the cochlea, as performed in this study, resulted in oblique and tangential sectioning of the spiral ganglion in much of segment 1, which will increase the intersubject variability of counting in this region.

The correlation of total spiral ganglion cell counts with cause of deafness demonstrated by statistical analysis in the present study is consistent with results of previous histopathologic studies,[3,5] but differs from the conclusions of Hinojosa et al[2,7,16] demonstrating no relationship between clinical diagnosis and numbers of remaining spiral ganglion cells.

There was considerable variability of total spiral ganglion cell counts within each diagnostic category in which there were significant numbers of remaining spiral ganglion cells (Table 2). The mean total spiral ganglion cell count has been estimated to be approximately 35,000 in normal-hearing young individuals and 29,000 in normal-hearing adults.[5] An analysis of total spiral ganglion cell count in 16 individuals with normal hearing demonstrated a range of 29,802 to 38,352 with a mean of 33,623.[17] With a mean of 28,418 for normal individuals (Table 2), even in the diagnostic category with the lowest mean spiral ganglion cell count (diagnostic group 1, postnatal viral labyrinthitis), the total spiral ganglion cell count was approximately 28% of normal. In the diagnostic groups with the highest mean total spiral ganglion cell count (categories 3 and 10, sudden idiopathic deafness and aminoglycoside ototoxicity), the remaining spiral ganglion cell counts were over 75% of normal. Although these data would suggest an adequate number of spiral ganglion cells for cochlear implantation in most deaf individuals, the findings must be tempered by at least two factors. First, the minimum requisite number of functioning spiral ganglion cells and the optimal distribution along the length of the cochlea of these cells is as yet undetermined. Second, the presence of a spiral ganglion cell at a light microscopic level does not guarantee normal physiologic function during life. It is known that in some forms of degeneration of first order cochlear neurons of the human organ of Corti, the loss of dendritic fibers may greatly exceed loss of ganglion cell bodies.[18] Thus the success of electrical stimulation may be different if stimulation is initiated at the dendritic level rather than at the level of the cell body. Nevertheless, the preservation of large numbers of spiral ganglion cells in most causes of profound deafness is reason for modest optimism about success of electrical stimulation of the inner ear.

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THE DEAFNESS RESEARCH FOUNDATION
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The Deafness Research Foundation invites applications for 1990 grant support of new research that promises to advance our understanding of the causes, treatment, and prevention of deafness and related ear disorders. The grants will be awarded to tax-exempt institutions for projects that will begin January 1, 1990, with the principal investigator of each project a member of the staff or faculty of such institution. The grants do not exceed $15,000, and can be competitively renewed with the same limit for an additional 1 or 2 years. Applications are reviewed for scientific merit of the proposed investigations and for their direct or potential clinical importance. The deadline for first year applications is July 15, 1989, and for renewal applications is August 15, 1989. For full information on the grant program, and for the required application form, please write to Walter A. Petryshyn, MD, Medical Director, The Deafness Research Foundation, 9 East 38th Street, New York, NY 10016; (212) 684-6556.

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MINOR CONGENITAL LARYNGEAL CLEFTS: DIAGNOSIS AND CLASSIFICATION

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Cleft larynx is a rare congenital anomaly. Detection of an unsuspected minor cleft may be difficult, but the pediatric laryngologist should suspect the possibility of cleft larynx from the clinical features. Four minor clefts are reported, three cases of supraglottic interarytenoid cleft and one of partial cricoid cleft. The technique for endoscopic diagnosis and the distinctive features are described and a classification into four types is proposed.

KEY WORDS — cleft larynx.

The embryologic pathogenesis of laryngotracheal clefts has been reviewed in detail by others.[1-3] The trachea and the esophagus at first share a common lumen, soon separated by the tracheoesophageal septum, which is normally complete by 35 days' gestation. Failure of the interarytenoid tissues or the cricoid cartilage[3] to fuse results in a laryngeal cleft. Partial or complete failure of formation of the tracheoesophageal septum produces a laryngotracheal cleft. Minor supraglottic clefts are due to absence of the interarytenoid soft tissues and musculature.

CLINICAL FEATURES

Patients present with signs of aspiration or partial airway obstruction. Aspiration causes coughing, choking, and cyanotic attacks, usually precipitated by feeding. There may be recurrent respiratory infections and aspiration pneumonitis with segmental collapse seen on the chest radiograph. Stridor due to partial obstruction of the larynx may be the presenting symptom in some patients and hamartomatous lesions associated with an unfused cricoid cartilage[3,4] may contribute to the stridor. An erroneous diagnosis of laryngomalacia may be made.[5] In some cases there is a weak, abnormal cry.

Most patients with clefts have other anomalies; esophageal atresia with or without tracheoesophageal fistula and gastroesophageal reflux are the most common.[2,6] Other associated defects[7] include cleft lip and palate; congenital heart defects; gastrointestinal anomalies, particularly of the anus; genitourinary anomalies, particularly hypospadias; and minor tracheobronchial anomalies. Laryngeal cleft occurs in two named syndromes.[7] G syndrome (or Opitz-Frias syndrome) consists of hypertelorism, hypospadias, cleft lip and palate, and cleft larynx. Pallister-Hall syndrome consists of congenital hypothalamic hamartoblastoma, hypopituitarism, imperforate anus, postaxial polydactyly, and cleft larynx.

CLASSIFICATION

At least two classifications have been proposed. In Petterson's[8] classification type 1 cleft extended through the cricoid lamina, type 2 extended lower and involved part of the tracheoesophageal wall, and type 3 extended to the carina. Evans[5] proposed a different classification. Type 1 was confined to the interarytenoid region above the level of the vocal cords and did not involve the cricoid cartilage. Type 2 included the cricoid cartilage and extended caudally to involve the cervical trachea. Type 3 extended to the thoracic trachea.

In the discussion we will propose an extension of these classifications to four types. "Minor" clefts are now more commonly recognized, the patients have a better prognosis, and the surgical approach is different from that for "major" clefts.

CASE REPORTS

Case 1. A full-term infant, birth weight 2,775 g, required intubation shortly after birth for bradycardia and apnea. Esophageal atresia with lower pouch tracheoesophageal fistula was confirmed and repaired. Extubation was accomplished 4 days later but there was repeated aspiration with oral feedings. At endoscopy a stricture of the esophageal anastomosis was dilated and a right tracheal bronchus identified. No residual or recurrent tracheoesophageal fistula or laryngeal cleft was noted. A gastrostomy was performed for persistent aspiration. Multiple vertebral and ureteral anomalies were present and the patient was diagnosed as having the VATER syndrome.[9]

There were four subsequent admissions for cough, respiratory infections, and difficulty with

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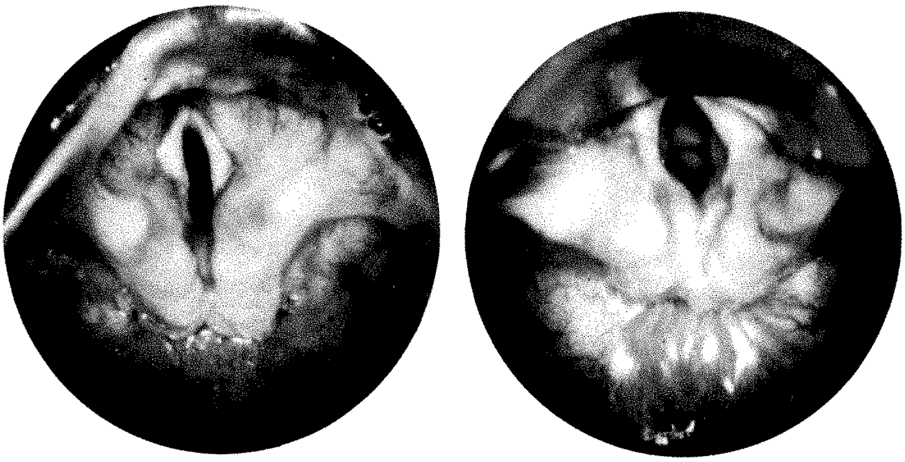

Fig 1. (Case 1) Direct laryngoscopic findings. Laryngoscope is used to splay vocal cords, stretch posterior commissure, and confirm supraglottic interarytenoid cleft (type 1).

feeding but the episodes became less severe and less frequent. Endoscopy at 3 years of age finally revealed an interarytenoid cleft, not extending below the vocal cords (Fig 1). No plans for closure of the cleft have been made as the pulmonary problems have improved with age. This case demonstrates the difficulty in identifying a minor cleft despite the known association of tracheoesophageal fistula that should alert the endoscopist to the possibility of a cleft larynx.

Case 2. A full-term infant weighing 2,900 g, born of a pregnancy complicated by moderate polyhydramnios, had respiratory distress and grunting stridor shortly after birth. Esophageal atresia and lower pouch tracheoesophageal fistula were repaired and a gastrostomy performed. The postoperative course was complicated by breakdown of the esophageal anastomosis requiring cervical esophagostomy. The patient had persistent cough, apneic attacks, and cyanosis. Endoscopy confirmed severe tracheomalacia. The larynx was reported to be normal. Many attempts at extubation over the next 2 weeks failed and tracheopexy[10] for the tracheomalacia was performed. This relieved the respiratory symptoms and extubation was successful.

A staged gastric tube reconstruction was performed at 2 years of age but the anastomosis required open revision and multiple esophageal dilatations. At one of the dilatations interarytenoid cleft to the level of the vocal cords was detected. The vocal cord movements were normal. The patient had no clinical signs of aspiration. The cleft caused no symptoms and operative repair was not contemplated.

Case 3. This child, born of a normal pregnancy and of a normal birth weight, had an imperforate anus that required a transverse colostomy. Recurrent coughing and choking episodes with feeding required endoscopy at 3 weeks of age and revealed a laryngeal cleft extending 4 mm below the level of the vocal cords (Fig 2). The mucosal part of the cleft did not extend to the inferior part of the cricoid cartilage. The patient could not tolerate oral feedings and was maintained at home on gastric tube feedings until 3½ months of age, when he was readmitted with gastroesophageal reflux, episodes of coughing after feedings, and failure to thrive. There was one episode of aspiration and cyanosis. At 6 months of age endoscopic closure of the glottic and subglottic portions of the cleft was attempted by deepithelializing the opposing surfaces of the

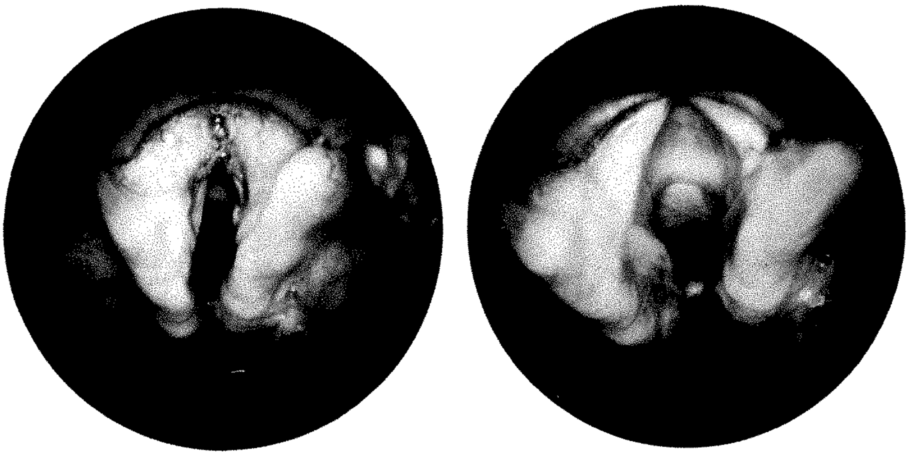

Fig 2. (Case 3) Direct laryngoscopic findings. Laryngoscope opens posterior glottis. Cleft extends about 4 mm below glottic level, constituting partial cricoid cleft (type 2).

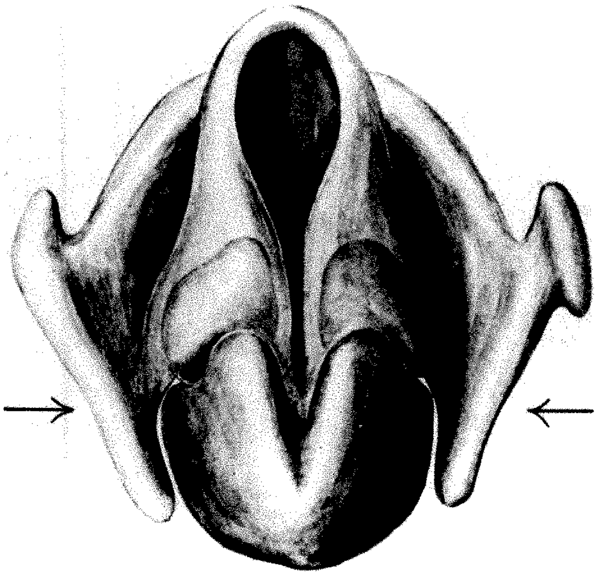

Fig 3. Artist's reconstruction of specimen showing partial cleft of cricoid cartilage (type 2) (arrows).[11]

cleft and suturing them together. At first oral feedings were well tolerated without aspiration. Laryngoscopy 2 weeks later showed closure of the cleft, but 1 month later, after an episode of vomiting and aspiration, further laryngoscopy showed breakdown of the repair. The closure was repeated and a Nissen fundoplication and gastrostomy was performed subsequently. The nutritional status rapidly improved and the patient was discharged at 10 months of age. Repeat laryngoscopy done 4 months later showed a pinpoint fistula at the lower end of the repair but there was otherwise good closure of the cleft for about 8 mm, to a level of 4 mm above the vocal cords.

Case 4. A child born of a normal pregnancy and of a normal birth weight had dysmorphic features including right epicanthal fold, low-set ears, clinodactyly and brachydactyly of the hands, and syndactyly of the fourth and fifth toes bilaterally. There were attacks of cough and cyanosis after feeding with weight loss and a right lower lobe pneumonia. At 3 months aspiration during swallowing was confirmed radiographically. Endoscopy showed a supraglottic interarytenoid cleft.

The nutritional status gradually improved on thickened feedings. Repeat endoscopy at 7 months of age confirmed the cleft. At 15 months of age there is still occasional aspiration but operative repair is not yet considered necessary.

DISCUSSION

In Evans's classification type 2 cleft is a cleft of the cricoid cartilage with extension caudally into the cervical trachea, but G. F. Tucker, Jr (personal communication) and Holinger et al[11] have pointed out that the cricoid cartilage may be only partly deficient (Fig 3).

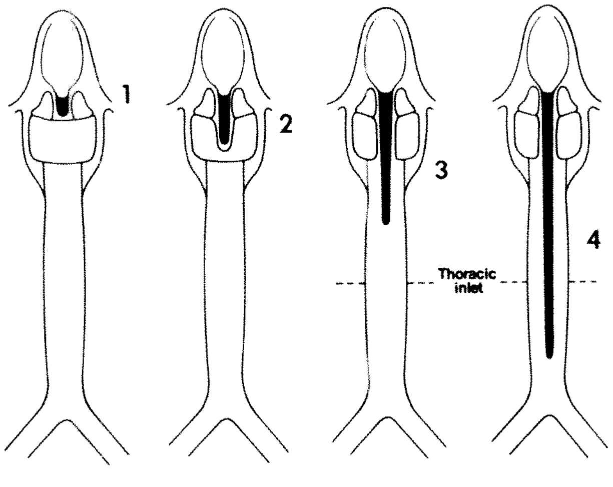

Fig 4. Diagram showing classification of four types of cleft larynx.

Our new classification incorporates the observation of Tucker and Holinger et al and subdivides Evans's type 2 while retaining his first and last types. The new classification we propose defines four types of laryngeal cleft (Fig 4). Type 1 is a supraglottic interarytenoid cleft. The cleft is above the level of the vocal cords (Fig 1). Type 2 is a partial cricoid cleft, extending below the level of the vocal cords and partly but not completely through the posterior lamina of the cricoid cartilage (Fig 3). There may be a mucosal bridge covering the partly absent cricoid arch (Fig 2).

Type 3 is a total cricoid cleft, extending completely through the cricoid cartilage with or without further extension into part of the cervical tracheoesophageal wall. Type 4 is a laryngoesophageal cleft. It involves a major part of the intrathoracic tracheoesophageal wall. A complete extension to the carina leaves a common esophageal and tracheal lumen.

It seems from the cases reported so far[1,3,5,12] that minor clefts (types 1 and 2) do not require tracheotomy, have a good prognosis, and can be managed conservatively or by endoscopic microsurgical repair. We have repaired a minor cleft in three patients (one reported above and two others) using the endoscopic technique described. On the other hand, major clefts (types 3 and 4) that include part or all of the tracheoesophageal wall require major transcervical or transthoracic surgery for closure and have a poor prognosis. When repair of a cleft is being considered, gastroesophageal reflux must be treated, if necessary by fundoplication.[13]

A specific endoscopic technique for diagnosis of cleft larynx should be part of every endoscopic examination of patients with aspiration and stridor.

Various techniques have been suggested, including examination of the postcricoid and upper esophageal region with a large endotracheal tube to splay the posterior glottis[14] or probing in the interarytenoid region with an instrument in each hand while the laryngoscope is in suspension.[3] Our technique[15] is to use a suitable laryngoscope such as the Holinger anterior commissure pediatric laryngoscope to deliberately separate the posterior glottis and examine the posterior commissure (Figs 1 and 2). An "occult" or "submucous" cleft occurs when the cartilage or muscle is deficient in the midline posteriorly but the mucous membrane is intact.

The examination is done with the patient under general anesthesia[16] and breathing spontaneously, a technique in which no endotracheal anesthesia tube is used.

Others[17] have pointed out that large clefts (types 3 and 4) involving the tracheoesophageal wall may be missed because of difficulty in visualization caused by an unstable, collapsing larynx and redundant esophageal mucosa, especially when the cleft has not been suspected clinically. It has been suggested[17] that cineradiographic studies with contrast be performed prior to endoscopy, but it is our experience that aspiration of contrast material can represent a significant hazard. We usually do not recommend contrast studies but proceed directly to endoscopy in the investigation of repeated aspiration.

A laryngeal cleft may be found in patients with the VATER association,[9] with tracheoesophageal fistula,[9,12] and with G syndrome.[18]

It is emphasized that although the clinical features of a supraglottic interarytenoid cleft may suggest the presence of laryngomalacia, final definitive diagnosis of the nature and degree of the anomaly can be made only by careful examination of the larynx with particular attention to the interarytenoid region.

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ANATOMIC FORAMINA IN THE THYROID CARTILAGE: INCIDENCE AND IMPLICATIONS FOR THE SPREAD OF LARYNGEAL CANCER

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Anatomic openings in the posterosuperior portion of the thyroid lamina of the larynx were first described in the 19th century. In the present study of 121 coronally sectioned larynges, such openings were found in 47 (39%), appearing bilaterally in 15 and unilaterally in 32. In a separate study of 69 cadaver larynges, the openings were found in 39 (57%), appearing bilaterally in 16 and unilaterally in 23. In 51 laryngectomy specimens removed for squamous cell cancer and showing foramina, tumor was not observed to invade or traverse the foramen in a single case, even in those specimens with tumor overlying the foramen and displaying framework invasion at other sites. Invasion by cancer through the foramen appears to be prevented by a layer of fibroelastic tissue overlying the medial surface of the foramen, parallel to the inner surface of the thyroid lamina. This layer of fibroelastic tissue may represent perichondrium.

KEY WORDS — laryngeal anatomy, laryngeal cancer.

INTRODUCTION

Anatomic openings in the thyroid lamina were first described by Segond[1] in 1847 and have been the subject of numerous subsequent studies.[2-8] They are found in the posterosuperior portion of the lamina, inferior to the superior thyroid tubercle and anterior to the oblique line (Fig 1), and range in diameter from less than 1 mm to 8 mm.

The few studies that have evaluated the incidence of thyroid foramina vary widely in their conclusions. In Keen and Wainwright's[6] study of 133 larynges, such openings were found in 17%, appearing bilaterally in 5% and unilaterally in 12%. Zemlin et al,[8] in a study of 66 cadaver larynges, found foramina in 33%, appearing bilaterally in 9% and unilaterally in 24%. Jelisiejew and Szmurlo[7] found an overall incidence of 38% (19% bilateral, 19% unilateral) in 484 larynges, and Waldeyer[4] found an incidence of 60% in 100 larynges.

Segond[1] noted that the foramina may transit the superior laryngeal vessels (Fig 2) and a subdivision of the external branch of the superior laryngeal nerve, findings subsequently supported by Dilworth.[9] Jelisiejew and Szmurlo[7] found both vessels and nerve branches in 57% of foramina, blood vessels alone in 31.5%, and nerve branches alone in 6.5%.

Despite the relatively constant location and anatomic relationships of the foramina, their embryologic origin has not been well established. Keen and Wainwright[6] stated that they are "the result of incomplete union of the cartilaginous elements of the fourth and fifth pharyngeal arches, from which the lamina is derived." Frazer,[3] on the other hand, stated that the presence of a foramen "does not indicate the line of junction of a fourth and fifth arch element in the complete cartilage, but rather the failure of chondrification to involve the spot, possibly because the presence of a vessel or some other cause has kept the preliminary concentrations of mesoblast from taking place there." Jelisiejew and Szmurlo[7] believe that the foramen is the result of a persistent inferior branch of the superior thyroid artery during fetal development.

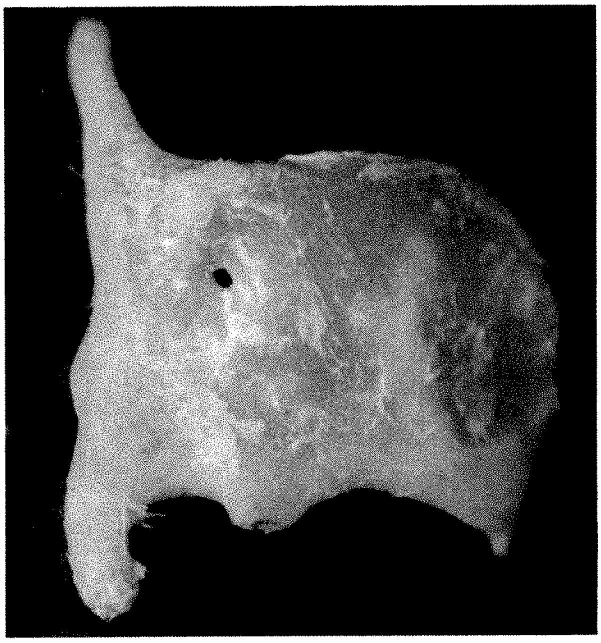

Fig 1. Thyroid ala with small foramen, typically located under base of superior cornu.

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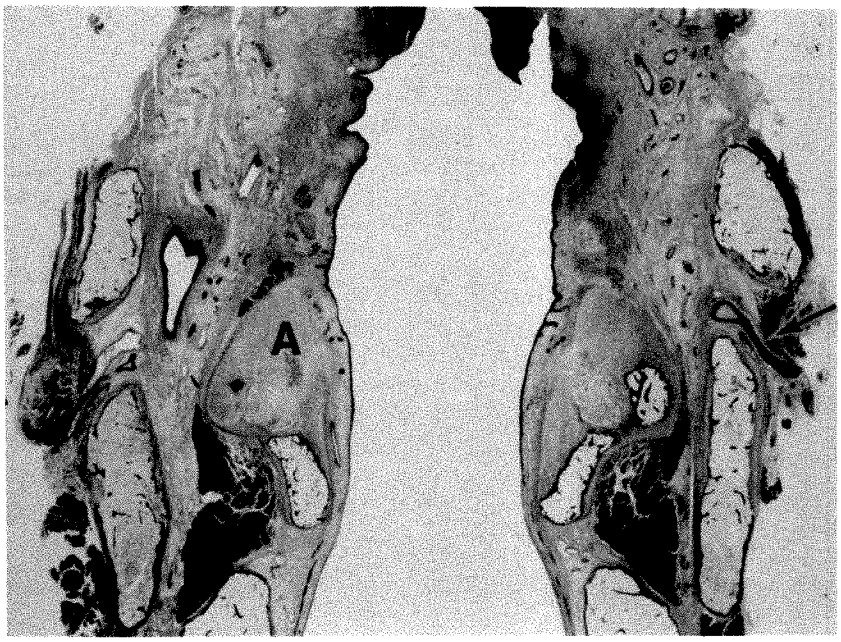

Fig 2. Coronal section through posterior larynx showing bilateral foramina, with blood vessel (arrow) traversing foramen on right. A — arytenoid cartilage.

The present study was prompted by the following case.

A 57-year-old man with a 3-cm diameter right jugulodigastric node was found on endoscopy to have a 1-cm diameter squamous cell carcinoma on the anterior wall of the right pyriform sinus. As the tumor did not involve the apex of the pyriform sinus, a right partial supraglottic laryngectomy, right functional neck dissection, and cricopharyngeal myotomy were performed. During elevation of the perichondrium from the posterosuperior portion of the thyroid lamina on the right side, an opening was encountered in the thyroid cartilage. As the opening was adjacent to the pyriform sinus lesion, a frozen section biopsy of soft tissue from within the opening was performed. The biopsy results were negative for tumor. Subsequent evaluation of the tumor through a pharyngotomy demonstrated that it was palpably free from the opening. Postoperative evaluation of the surgical specimen demonstrated that the opening was an anatomic thyroid foramen measuring 5 mm in diameter and containing no tumor.

Although tumor extension through the foramen was not observed in the case described above, extension of squamous cell carcinoma through the framework of the larynx has been associated with an increased incidence of nodal metastases and decreased survival.[10-12] As thyroid foramina are not uncommon, it would be of interest to know whether they may act as a pathway for the spread of laryngeal cancer into the extralaryngeal soft tissues.

The purpose of the present study was twofold: 1) to reevaluate the incidence and size of the anatomic foramina and 2) to determine whether they act as a pathway for the spread of tumor into the extralaryngeal tissues.

MATERIALS AND METHODS

Four hundred twelve surgical specimens removed for laryngeal cancer and 23 cadaver larynges, all processed in celloidin, were studied by the whole organ serial section technique. Sections were made at a thickness of 20 μm and stained with hematoxylin and eosin.

One hundred twenty-one coronally sectioned laryngectomy specimens (115 from men, six from women) were examined for the presence of thyroid foramina. Forty-six larynges with cancer and one normal larynx were found to contain foramina. The existence of each foramen was verified by ensuring that cartilage was present in sections both anterior and posterior to the sections showing the foramen, so as not to mistake the angle between the superior cornu and the upper border of the thyroid lamina for a foramen. The age of each patient was recorded, and in larynges with a unilateral window, the side was recorded.

The coronal dimension of each window was measured directly from the slides, with a range of error of ±0.5 mm. Horizontal dimensions of the

INCIDENCE OF ANATOMIC THYROID FORAMINA

Coronally sectioned laryngectomy specimens (n = 121)			
Larynges with foramina			47 (39%)
Bilateral foramina		15 (12%)	
Unilateral foramina		32 (27%)	
Right-sided	11 (9%)		
Left-sided	21 (18%)		
Larynges without foramina			74 (61%)
Cadaver larynges (n = 69)			
Larynges with foramina			39 (57%)
Bilateral foramina		16 (24%)	
Unilateral foramina		23 (33%)	
Right-sided	12 (17%)		
Left-sided	11 (16%)		
Larynges without foramina			30 (43%)

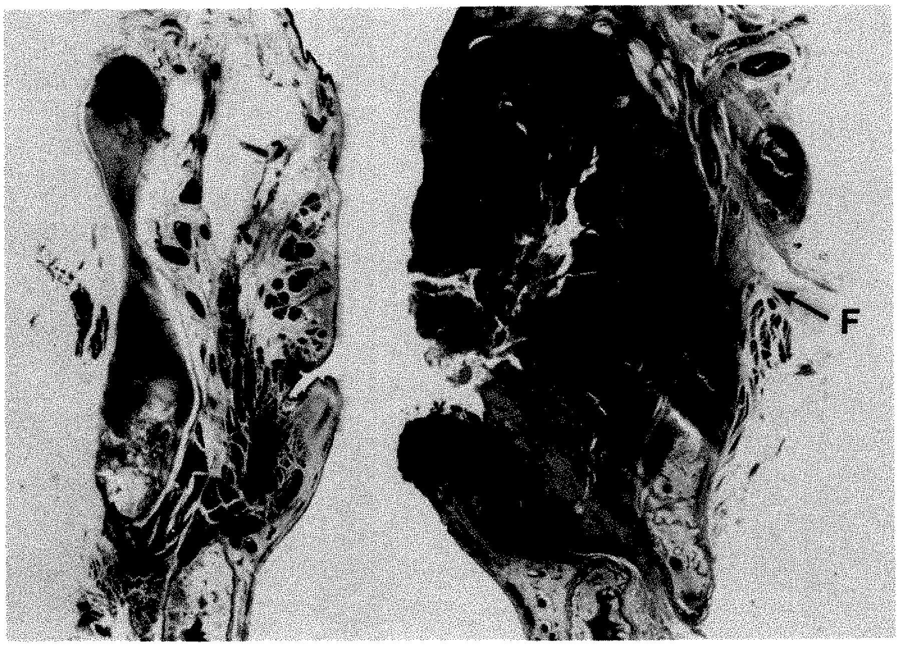

Fig 3. Coronal section of transglottic cancer through midposterior larynx, showing tumor overlying but characteristically not penetrating foramen (F).

foramina could not be assessed accurately, for two reasons. First, only every 100th section had been stained. The stained sections were thus approximately 2 mm apart, a distance greater than the diameter of some of the foramina. Second, imperfectly cut specimens were not included in the numbering process. In the cadaver portion of the study described below, the openings were noted to be circular or oval in cross-sectional configuration.

Sixty-nine cadaver larynges were examined to determine whether the incidence figures were comparable to those recorded from the sectioned specimens. Data collected included the presence or absence of openings and the side involved if unilateral.

A total of 190 larynges were thus examined for the initial portion of the study, consisting of 121 total laryngectomy specimens and 69 cadaver specimens.

Of the 121 coronally sectioned total laryngectomy specimens, 45 had both a squamous cell carcinoma and either unilateral or bilateral foramina. One had an adenocarcinoma and a unilateral thyroid foramen. These 46 specimens were examined for tumor extension into or through the foramina. In addition, six coronally sectioned hemilarynges were evaluated. Each contained a squamous cell carcinoma and displayed a foramen, but was not included in the incidence portion of the study because only one thyroid lamina had been sectioned. Of the total of 52 laryngeal tumors, eight were supraglottic, 17 glottic, 16 transglottic, and 11 pyriform sinus. In 17 of the 52, framework invasion had occurred on the same side as the foramen.

RESULTS

Data concerning the incidences of thyroid foramina in the laryngectomy specimens and cadaver larynges are shown in the Table. The vertical dimensions of the foramina ranged from less than 1 mm to 6 mm, averaging 2.5 mm (standard deviation, ±1.2 mm). The average age of patients with foramina was 61.4 years, and without foramina 61.9 years.

In the laryngectomy specimens and cadaver larynges with unilateral openings, they were observed on the right side in a total of 23 cases and on the left side in a total of 32 cases.

In only one specimen, the larynx with adenocarcinoma, was tumor observed to pass through the foramen. In none of the 51 larynges with squamous cell carcinoma was tumor observed either to pass through the foramen or to invade cartilage around the foramen unless extensive invasion of adjacent posterior or inferior thyroid cartilage had already occurred. This observation was true even in 13 larynges displaying invasion of nonadjacent portions of the laryngeal framework on the same side. It was also true of 12 larynges in which tumor directly overlay, or was within 2 mm of, the foramen (Fig 3).

Tumor extension toward the foramina appeared to be limited by a layer of fibroelastic tissue covering the medial surface of the openings, continuous with the inner perichondrium of the thyroid cartilage (Fig 4). A separate layer overlying the lateral surface was also visible in some specimens, particularly those with smaller foramina. Also noticeable, particularly in the smaller foramina, was loose connective tissue between the layers of fibroelastic tissue.

DISCUSSION

The incidence rates for thyroid foramina in the

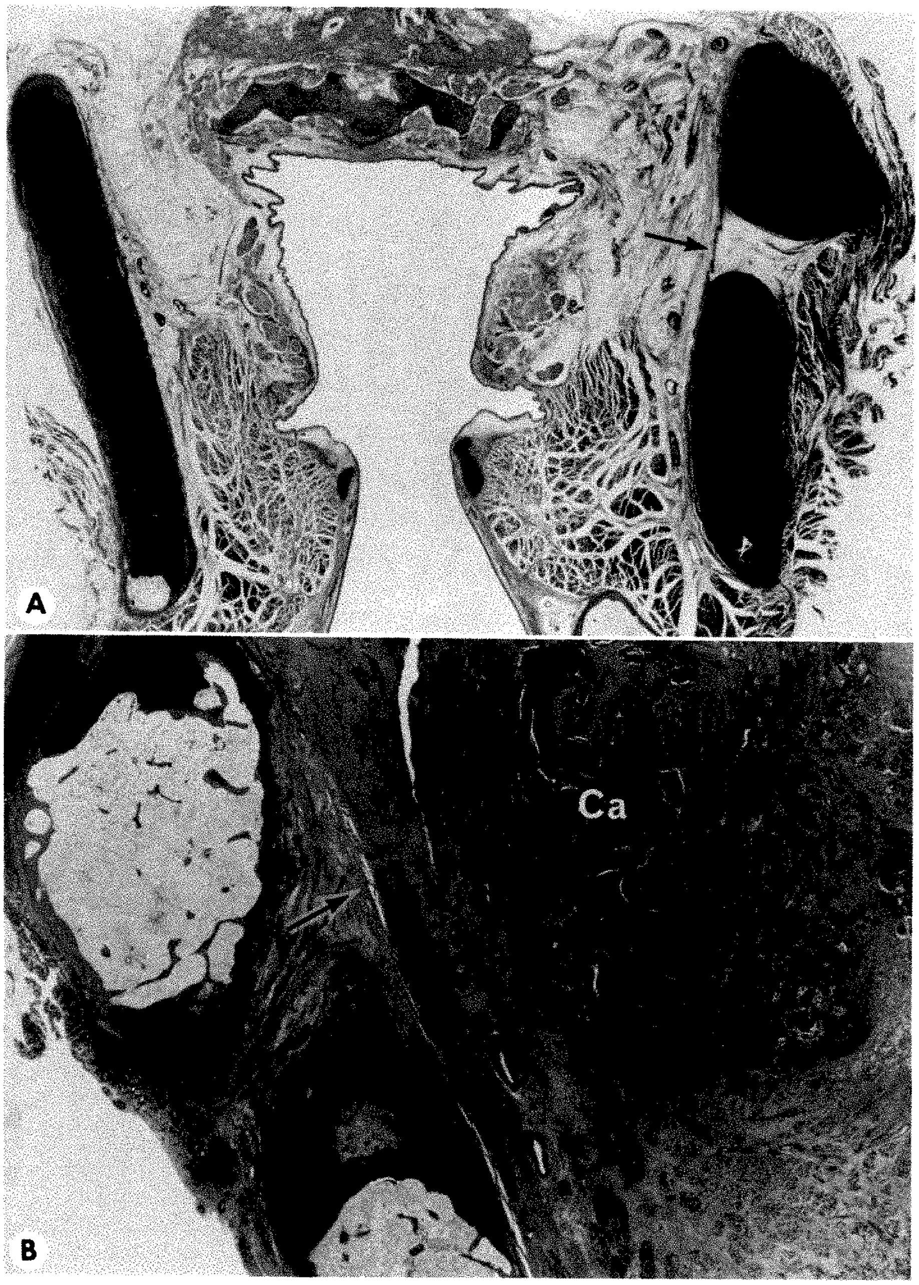

Fig 4. Coronal sections. A) Through mid-posterior larynx. Layer of fibroelastic tissue (arrow) overlies medial aspect of foramen in this and all other specimens examined. B) Through thyroid foramen in larynx with pyriform sinus cancer. Tumor (Ca) does not extend lateral to layer of fibroelastic tissue (arrow) overlying medial aspect of foramen.

present study are comparable to the higher rates noted by Jelisiejew and Szmurlo[7] and by Waldeyer.[4]

The incidence data recorded from the laryngectomy specimens probably underestimate the true incidence of foramina in the sectioned larynges, because the distance between sections averaged approximately 2 mm, greater than the diameter of the smaller openings. Openings less than 2 mm in horizontal dimension might be overlooked in histologic sections but would be detectable on gross specimens. Ten serially sectioned larynges with deep cartilage indentations in the posterosuperior quadrant of the thyroid lamina, suggesting the possible presence of a foramen in a slightly anterior or posterior plane, could not be included in the tabulations because additional, closely adjacent sections were not available for study.

The average vertical dimension of the foramina noted in the present study of larynges predominantly from men is comparable to the average diameter of 2.7 mm noted in men by Jelisiejew and Szmurlo.[7] Our finding that unilateral foramina are somewhat more common on the left side is also in accord with the results of previous studies.[6-8]

The observation that the foramina do not serve as preformed pathways either for tumor invasion of the laryngeal framework or for the spread of even aggressive squamous cell tumors into the extralaryngeal soft tissues is significant. Although the foramina may be engulfed during broadly advancing framework invasion by pyriform sinus or transglottic tumors, they appear to be as resistant to the spread of laryngeal cancer as the remainder of the thyroid lamina. Resistance to tumor spread appears to be conferred primarily by the well-defined layer of fibroelastic tissue overlying the medial surface of the foramen. This hypothesis is consistent with an earlier report from this laboratory[13] that the spread of laryngeal cancer is resisted by the internal perichondrium of the thyroid cartilage until late in its growth.

The findings are of surgical significance for two reasons. First, as laryngeal surgery for benign lesions and phonatory disorders may involve exposure of one or both thyroid alae, and as the contents of the foramina may consist of the superior laryngeal vessels and a subdivision of the external branch of the superior laryngeal nerve, a knowledge of the incidence and location of these foramina is important so that their contents may be preserved.

Secondly, it is important for the surgeon to know that squamous cell carcinoma does not escape from the larynx through these preformed openings. This observation, together with the fact that supraglottic cancer has not invaded the thyroid alae in any of our 93 specimens (unpublished data), has important surgical implications. Since thyroid cartilage invasion by advanced cases of glottic and transglottic cancer starts at the inferior border,[14] and invasion by cancer of the pyriform sinus begins at the posterior border,[15] the surgeon who finds an isolated opening in the thyroid cartilage near the base of the superior cornu during conservation laryngectomy can feel confident that it does not represent tumor. Frozen section biopsy may still be desired for confirmation, but the results of the present study suggest that isolated invasion of squamous cell carcinoma through the posterosuperior portion of the thyroid ala is unlikely.

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Ann Otol Rhinol Laryngol 98:1989

BACTERIOLOGY OF CHRONIC MAXILLARY SINUSITIS IN ADULTS

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Aspirates of 72 chronically inflamed maxillary sinuses were processed for aerobic and anaerobic bacteria. Bacterial growth was present in 66 of the 72 specimens (92%). Anaerobic bacteria were isolated in 58 of the 66 culture-positive specimens (88%). Anaerobes only were recovered in 37 cases (56%) and in 21 (32%) they were mixed with aerobic or facultative bacteria. Aerobic or facultative bacteria were present in eight cases (12%). A total of 185 isolates (2.8 per specimen) — 131 (2.0 per specimen) anaerobes and 54 (0.8 per specimen) aerobes or facultatives — were isolated. The predominant anaerobic organisms were anaerobic cocci and *Bacteroides* sp, and the predominant aerobes or facultatives were *Streptococcus* sp and *Staphylococcus aureus*. Twelve of the 27 *Bacteroides* sp that were tested for β-lactamase (44%) produced the enzyme. These findings indicate the major role of anaerobic organisms in chronic sinusitis.

KEY WORDS — bacteriology, *Bacteroides* sp, *Peptostreptococcus* sp, sinusitis, *Staphylococcus aureus*.

INTRODUCTION

The microbiology of chronic maxillary sinusitis in adults has been shown to be polymicrobial, consisting of aerobic and anaerobic bacteria.[1-5] However, the recovery rate of anaerobic bacteria varied in these studies from 25% to 56%. The variability in recovery rate may be due to differences in the population studied and in techniques of transportation of specimens and cultivation of organisms.

The report by Frederick and Braude[3] established the importance of anaerobes in chronic sinusitis. Recent improvements in microbiology, transportation system, and changes in nomenclature of microorganisms and their resistance to penicillin stimulated us to summarize our recent experience. This study reports our experience in recovery of microorganisms over a period of 10 years in adults with chronic maxillary sinusitis.

PATIENTS AND METHODS

The study included 72 patients, 53 men and 19 women. Their ages ranged from 18 to 62 years (average, 28 years). All materials were obtained from consecutive patients who had undergone a Caldwell-Luc operation because of chronic maxillary sinusitis from June 1974 to June 1984. These procedures were performed in either Walter Reed Army Medical Center or the National Naval Medical Center. None of the patients received local or systemic antimicrobial therapy for at least 1 week prior to the operation. The course of the disease was of at least 3 weeks' duration. Patients who received antimicrobials were excluded from the analysis.

Chronic sinusitis was judged to be present if the radiographic studies showed mucosal thickening and either an air-fluid level or complete opacification of the maxillary sinus. All patients had tenderness over the maxillary sinus. Occipitomental (Waters' view), lateral, oblique, and verticomental views were obtained. The degree of mucosal thickening was evaluated by noting the nearest distance between the air-mucosal interface and the lateral part of the sinus wall. Mucosal thickening was defined as a mucosal width of 5 mm or more. Patients had at least one of these complaints: facial pain, frontal headache, purulent nasal discharge, fever, or malaise.

The otolaryngologist obtained the specimens during surgery, employing strict asepsis to avoid any contamination. Specimens were transported to the laboratory in a syringe sealed with a rubber stopper after evacuation of the air or in an anaerobic transport tube (Port-A-Cul, Baltimore Biological Laboratories, Cockeysville, Md). The time between the collection of materials and the inoculation of specimen was never more than 30 minutes for syringes and 3 hours for the anaerobic transport tube.

Specimens were inoculated onto 5% sheep's blood, chocolate, and MacConkey agar plates for aerobic and facultative organisms. The plates were incubated at 37°C aerobically (MacConkey) or under 5% CO_2 (5% sheep's blood and chocolate) and examined at 24 and 48 hours. For anaerobes the material was plated onto prereduced vitamin K_1-enriched *Brucella* blood agar, an anaerobic blood agar plate containing kanamycin and vancomycin, an anaerobic blood plate containing colistin and nalidixic acid, and an enriched thioglycolate broth (containing hemin and vitamin K_1).[6] The anaerobic plates and thioglycolate broth were incubated in GasPak jars (Baltimore Biological Laboratories, Cockeysville, Md) and examined at 48 and 96 hours.

Anaerobes were identified by techniques described previously.[6] Aerobic bacteria were identi-

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NUMBER OF BACTERIAL ISOLATES IN 66 PATIENTS WITH CHRONIC MAXILLARY SINUSITIS

Aerobic or facultative bacteria	
Gram-positive	
Staphylococcus aureus	9
Staphylococcus epidermidis	2
Streptococcus pyogenes	5
Group B β-hemolytic streptococci	3
Group C β-hemolytic streptococci	1
Group F β-hemolytic streptococci	2
α-Hemolytic streptococci	10
Streptococcus pneumoniae	4
Gram-negative	
Neisseria sp	3
Branhamella catarrhalis	4
Hemophilus influenzae	3
Hemophilus parainfluenzae	2
Escherichia coli	2
Klebsiella pneumoniae	1
Pseudomonas aeruginosa	2
Proteus sp	1
Total aerobic or facultative isolates	54
Anaerobic bacteria	
Gram-positive	
Peptostreptococcus sp	9
Peptostreptococcus micros	7
Peptostreptococcus asaccharolyticus	4
Peptostreptococcus morbillorum	2
Peptostreptococcus prevotii	4
Peptostreptococcus anaerobius	3
Peptostreptococcus magnus	8
Streptococcus constellatus	1
Streptococcus intermedius	1
Veillonella parvula	5
Actinomyces israelii	1
Eubacterium lentum	2
Propionibacterium acnes	19
Propionibacterium sp	3
Clostridium butyricum	2
Gram-negative	
Fusobacterium sp	4
Fusobacterium nucleatum	5
Fusobacterium necrophorum	1
Bacteroides sp	14
Bacteroides fragilis	2
Bacteroides vulgatus	1
Bacteroides thetaiotaomicron	1
Bacteroides melaninogenicus	15
Bacteroides intermedius	7
Bacteroides asaccharolyticus	6
Bacteroides oralis	2
Bacteroides ureolyticus	1
Bacteroides oris-buccae	1
Total anaerobic isolates	131

fied by conventional methods.[7] β-Lactamase activity was determined for all *Bacteroides* isolates during the last 4 years of the study, with use of the chromogenic cephalosporin analogue 87/312 method.[8]

RESULTS

Bacterial growth was present in 66 of the 72 specimens (92%). Anaerobic bacteria were isolated in 58 of the 66 culture-positive specimens (88%). They were the sole bacterial isolates in 37 cases (56%) and in 21 (32%) they were mixed with aerobic or facultative bacteria. Aerobic or facultative bacteria were present in eight cases (12%).

There were 185 bacterial isolates recovered from the 66 culture-positive specimens (2.8 per specimen) (see Table). One hundred thirty-one were anaerobes (2.0 per specimen), and they included the following, in descending order of frequency: anaerobic cocci (39 isolates), *Bacteroides* sp (50, including 28 *Bacteroides melaninogenicus* group and four *Bacteroides fragilis* group), *Propionibacterium acnes* (19), and *Fusobacterium* sp (ten).

There were 54 aerobic or facultative anaerobic isolates (0.8 per specimen), and they included the following in descending order of frequency: α-hemolytic streptococci (ten isolates), *Staphylococcus aureus* (nine), and *Streptococcus pyogenes* (five).

Twenty-seven of the isolates of *Bacteroides* sp were tested for production of β-lactamase, of which 12 (44%) were positive. These included three isolates of *B fragilis* group and nine of 17 isolates (53%) of *B melaninogenicus* group.

DISCUSSION

This report confirms the study of Frederick and Braude[3] that was done from 1960 to 1967. They isolated anaerobes in 69% of their culture-positive specimens; this report describes their recovery in 88% of our patients. These data are in accordance with the information we obtained from a study in children with chronic sinusitis, in which anaerobes were isolated in all 37 culture-positive patients.[9] In contrast to the study in children[9] and similar to that of Frederick and Braude,[3] our study shows *B fragilis* in 6% of our culture-positive specimens.

The frequent involvement of anaerobes in chronic sinusitis probably is related to the poor drainage and increased intranasal pressure that develops during inflammation.[10] This can reduce the oxygen tension in the inflamed sinus[11] by decreasing the mucosal blood flow[12] and depressing the ciliary action.[13] The lowering of the oxygen content and pH of the sinus cavity supports the growth of anaerobic organisms by providing them with an optimal oxidation-reduction potential.[13]

This report describes in greater detail than previous studies the species and subspecies of the different anaerobes involved in chronic sinusitis. The emergence of β-lactamase–producing *Bacteroides* sp other than *B fragilis* is an alarming phenomenon.

The resistance of *S aureus* and *B fragilis* to penicillin has been recognized previously and has influenced the choice of antibiotics for the therapy of infections involving these organisms. The growing number of β-lactamase–producing organisms belonging to the *B melaninogenicus* group, *Bacteroides oralis*, and *Fusobacterium* sp, however, has been noticed only in recent years.[14] These species are the predominant *Bacteroides* in the oral flora and are, therefore, those most commonly recovered in anaerobic infection in and around the oral cavity.[1] It may be that these organisms not only survive penicillin therapy but also protect penicillin-susceptible bacteria from penicillin by releasing the free enzyme into the abscess cavity.[15] Penicillin was considered the drug of choice for the therapy of such infection because of the susceptibility of most oral pathogens. The growing resistance of these strains, however, limited the use of this drug. We recently demonstrated the emergence of aerobic and anaerobic β-lactamase–producing organisms following one course of 7 to 10 days of penicillin therapy.[16] Because anaerobic bacteria are recovered frequently from chronic sinus aspirates, antimicrobial therapy should be directed also at the eradication of these organisms. Alternatives include clindamycin, chloramphenicol, cefoxitin, imipenem, metronidazole, and the combination of a β-lactamase inhibitor (clavulanic acid) and a penicillin (amoxicillin or ticarcillin). All these agents are resistant or indifferent to the enzyme β-lactamase.

The use of antimicrobial therapy alone without surgical drainage of collected pus may not result in clearance of the infection and may lead to the development of life-threatening complications such as intracranial abscesses.[17] The chronically inflamed sinus membranes with diminished vascularity may not carry an adequate antibiotic level to the infected tissue, even though the blood level may be therapeutic. Furthermore, the reduction in pH and oxygen tension within the inflamed sinus may further interfere with the activity of the antimicrobial agents and result in bacterial survival despite a high antibiotic concentration.[1,18]

The early detection of infection and initiation of appropriate antimicrobial therapy in sinusitis, with consideration of the probable presence of anaerobic organisms, is of utmost importance. Appropriate medical therapy during the initial phase of infection will reduce the likelihood of the complications of sinusitis and thereby decrease the morbidity and mortality of this potentially life-threatening infection.

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AUDIOMETRIC RESULTS OF TORP AND PORP MIDDLE EAR RECONSTRUCTION

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The results of 70 middle ear reconstructions using either total or partial ossicular replacement prostheses are presented, the mean observation period being 45 months. Twenty-seven of the prostheses (39%) were extruded, this occurring as late as 72 months after surgery, and four (6%) were removed at reoperation. The surgical results when assessed by the magnitude of the air-bone gap are comparable to those of other series in the literature. However, postoperative air conduction thresholds remained high, and 23% of the patients are using hearing aids. The average air-bone gap for the frequencies 0.5, 1, and 2 kHz does not provide information on patients' everyday hearing level, and should no longer be employed as an index for assessing the functional results of middle ear surgery.

KEY WORDS — air-bone gap, audiometric results, functional hearing, partial ossicular replacement prosthesis, speech frequencies, total ossicular replacement prosthesis, tympanoplasty.

The surgical management of grossly diseased middle ears is fraught with difficulties, and the results of reconstruction are not infrequently discouraging. Although innumerable studies involving many different reconstruction techniques have been published since the pioneer papers of Zöllner[1] and Wullstein,[2] the success rate has not always lived up to expectations.[3] The first ossicular prostheses were of polyethylene,[2,4] but were later found to provoke an inflammatory response resulting in extrusion.[5,6] A similar sequence of events, initial enthusiasm soon to be tempered by disappointing long-term results, has since been a familiar pattern with other alloplastic materials.

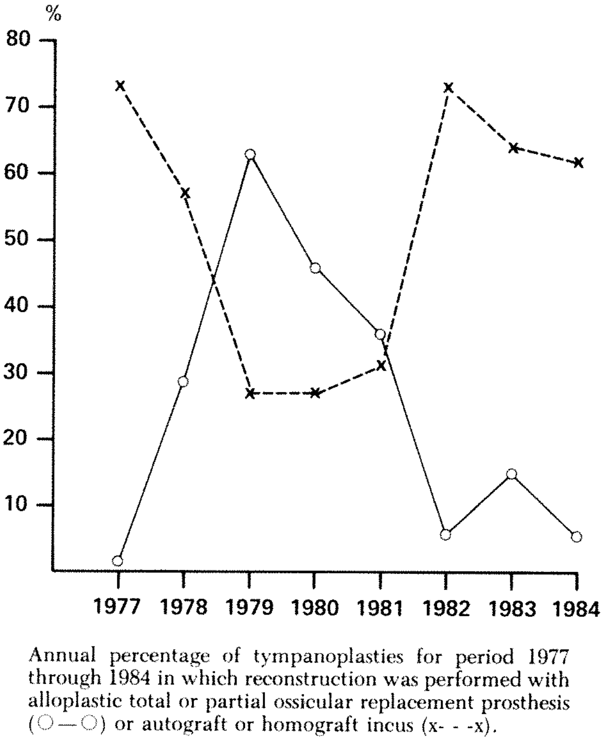
In the middle 1970s, the total and partial ossicular replacement prostheses (TORP and PORP) made of Proplast and Plastipore were introduced, and encouraging short-term results were claimed for these more biocompatible materials.[7,8] Although satisfactory results with respect to dry ears and closure of the air-bone gap have been reported subsequently,[9-11] some authors, particularly European, have reported an unacceptably high extrusion rate with a disappointing audiometric outcome.[12-15]

This report presents a follow-up study of a series of TORP and PORP operations and a discussion of the methods of presenting audiometric results.

MATERIAL AND RESULTS

The case records of all tympanoplasties, excluding myringoplasty, performed in the 8-year period 1977 through 1984 were reviewed in 1987. There were 314 tympanoplasties, in 70 of which either TORP or PORP reconstructions were performed, the former being used in ears lacking a stapes superstructure, while the PORPs were positioned on an intact stapes. These prostheses reached a peak frequency in 1979, being used in 63% of all tympanoplasties, but were employed infrequently after 1981. The relative frequency with which either TORPs and PORPs or autograft and homograft ossicles were employed is shown in the Figure, and reveals an almost inverse relationship; the remaining procedures were either different versions of type II or type III or type IV tympanoplasties.

Thirty-seven TORPs and 33 PORPs were inserted in 65 ears in 64 patients, five ears being reoperated



Annual percentage of tympanoplasties for period 1977 through 1984 in which reconstruction was performed with alloplastic total or partial ossicular replacement prosthesis (○—○) or autograft or homograft incus (x- - -x).

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TABLE 1. PROSTHESIS COVERING AND EXTRUSION

Prosthesis Covering		*Prosthesis Extrusion*		
Type	*No.*	*Mean Interval (mo)*	*No.*	*%*
None	24	27	18	75
Cartilage	17	31	3	18
Perichondrium	29	33	6	21
Total	70		27	39
Mean		29		

on with these prostheses during the period under consideration. Although staging has not been the treatment philosophy, a total of 151 operative procedures of all types have been performed on these 65 ears both prior to and including the period under consideration. Only 22 ears have been operated on once, 25 twice, while the maximum number of procedures per ear was six. Temporalis fascia was used for tympanic membrane reconstruction. Pars tensa perforations were found in 29 of the procedures (41%), pars flaccida perforations in 11 (16%), and cholesteatoma in 30 (43%). Radical mastoidectomy had been performed at an earlier date in 14 ears and was combined with reconstruction in a further 20. A radical cavity was thus the end result in 34 of the 65 ears (52%).

The mean postoperative follow-up was 45 months, with a range from 1 month in one of the reoperated ears to 8 years. Twenty-seven of the prostheses (39%) were extruded and four (6%) were removed at reoperation. Extrusion occurred in 41% and 36%, respectively, of the TORP and PORP procedures. Interposition of cartilage or perichondrium between the prosthesis and the tympanic membrane was first adopted in 1979 and employed routinely thereafter. This resulted in a reduction of extrusion frequency from 75% to 20%, but did not significantly influence the time (mean interval of 29 months) at which this occurred (Table 1). Spontaneous extrusion has so far occurred as late as 72 months after operation. Fourteen ears (21%) have been reoperated on with procedures not involving TORPs or PORPs. Postoperative atelectasis occurred in 19 ears (29%), and varying degrees of otorrhea after 21 (30%) of the procedures. There were no statistically significant differences between TORP and PORP procedures.

The air-bone gap was obtained by subtracting the lowest recorded bone conduction threshold from the postoperative air conduction threshold at each frequency. The results are presented in Table 2 in 5-dB steps from 0 to 30 dB at octave intervals from 0.25 to 4 kHz, the measure being percentage of all 70 operative procedures. The magnitude of the air-bone gap is frequency dependent. At the 10-dB criterion level, the percentages vary from a low of 20% at 0.25 kHz to a high of 49% at 2 kHz, the latter frequency maintaining "best" results at all levels, with 83% of TORP and PORP operations resulting in an air-bone gap of 30 dB or less.

TABLE 2. POSTOPERATIVE AIR-BONE GAP IN 5-dB STEPS EXPRESSED AS PERCENTAGE OF OPERATIVE PROCEDURES PERFORMED (n = 70)

Air-Bone Gap (dB)	*At 0.25 kHz*	*At 0.5 kHz*	*At 1 kHz*	*At 2 kHz*	*At 4 kHz*	*At 0.5, 1, and 2 kHz*
0- 5	9	11	16	30	21	19
0-10	20	21	26	49	29	35
0-15	27	30	33	59	41	43
0-20	37	39	41	73	47	52
0-25	49	50	54	79	54	58
0-30	60	56	64	83	69	71

In Table 3 are shown the mean preoperative and postoperative air conduction thresholds for the 70 ossicular reconstructions. Surgery had resulted in improvements in these mean thresholds ranging from 2 to 5.8 dB at 4 and 0.25 kHz, respectively, and a threshold deterioration of 2 dB at 8 kHz. The mean preoperative air conduction thresholds were 11 to 14 dB lower in the PORP ears, but the postoperative threshold changes were of the same order of magnitude in both groups. The mean postoperative air conduction threshold was maximally 5 dB lower in ears in which the prostheses were retained. The possible influence of a variety of factors was examined, but the hearing results were not influenced significantly by the degree of mastoid pneumatization, the extent of middle ear mucosal changes, the presence of cholesteatoma, the occurrence of otorrhea and atelectasis, or the creation of a radical mastoid cavity. Fifteen patients (23%) are now using hearing aids, eight of whom had received TORPs, and seven PORPs.

DISCUSSION

The results of middle ear reconstructive surgery are influenced by the choice of audiometric data for evaluation.[16,17] Since the aim of surgery is correction of a mechanical obstruction, it is perhaps natural that attention has been focused on the residual conductive deficit after operation, the air-bone gap. Traditionally, the air-bone gap is computed as the mean for the so-called speech frequencies of 0.5, 1, and 2 kHz, with the preoperative bone conduction providing the reference, since this had been the standard method for reporting the results of otosclerosis surgery. However, bone conduction thresholds are influenced by the impedance characteristics of

TABLE 3. MEAN PREOPERATIVE AND POSTOPERATIVE AIR CONDUCTION THRESHOLDS AT OCTAVE FREQUENCIES FROM 0.25 TO 8 kHz (n = 70)

Frequency (kHz)	*Air Conduction Threshold in dB*		*Difference (dB)*
	Preoperative	*Postoperative*	
0.25	50.1	44.3	−5.8
0.50	49.1	44.6	−4.5
1.00	47.6	43.4	−4.2
2.00	44.4	40.1	−4.3
4.00	55.3	53.3	−2.0
8.00	53.0	55.0	+ 2.0

Minus — improvement, plus — deterioration.

TABLE 4. POSTOPERATIVE AVERAGE (0.5, 1, 2 kHz) AIR-BONE GAP EXPRESSED AS PERCENTAGE OF CASES

Air-Bone Gap (dB)	*Jackson et al*[9] *(n = 55)*	*Brackmann et al*[10] *(n = 407)*	*This Study (n = 70)*
0- 5		28	19
0-10	22	50	35
0-15		63	43
0-20	49	73	52
0-25		79	58
0-30	80	85	71

For this study, results are combined for partial and total ossicular replacement prostheses. For other two studies, only results for partial are used.

the middle ear[18] and may therefore change as a result of surgically altered middle ear transmission. This can result in the well-known and physiologically unrealistic phenomenon of "overclosure" of the air-bone gap in successful stapes surgery.[17,19] Recognition of this fact has led to the use of either the postoperative[9] or the best, ie, lowest,[10,11] bone conduction threshold for obtaining the air-bone gap. In many publications the method with which the air-bone gap has been obtained is not described, thus making comparison of results even less meaningful.

The classical three-frequency pure tone average is not an accurate representation of the functional outcome of surgery since the frequencies 0.5, 1, and 2 kHz do not make equal contributions to speech perception.[20] Skewed thresholds may produce identical averages, but the functional result will, for example, not be the same in ears with predominantly 0.5- or 2-kHz improvements. Furthermore, this method of evaluation ignores the importance of auditory function at higher frequencies, 2 to 5 kHz, for social hearing.[20-22] The term speech frequencies, therefore, should no longer be restricted to 0.5, 1, and 2 kHz in the otosurgical literature. Hearing thresholds at 4 and 8 kHz are reported less often, but sensorineural loss at these frequencies is a recognized complication of chronic ear surgery[23,24] and will adversely affect patients' social hearing. Even a simple myringoplasty can produce higher thresholds at these frequencies.[25] It recently was reported that significant threshold losses occur in the high frequency range of 8 to 20 kHz following both myringoplasty and stapes surgery, and are probably an indication of iatrogenic inner ear trauma.[26] More subtle degrees of operative damage to sensory cells in the lower, speech frequencies cannot be excluded, since threshold change is an unreliable measurement of the extent of peripheral auditory lesions.[27]

The present report comprising only 70 surgical procedures cannot compete numerically with the largest in the literature, 1,042 operations,[10] but is of the same order of magnitude as the majority of studies, which have varied from 29 to 144 cases.[6,9,11-15,28] This relatively restrictive use of these prostheses is possibly symptomatic. The results of all procedures performed have been included in the present analysis irrespective of whether the prostheses have been extruded or the ears reoperated on; this fact accounts for the shortest observation periods.

In spite of early claims of biocompatibility,[7,8] inflammatory reaction with rejection and microdisintegration of these prostheses has been a problem.[14,15] Recognition of the high extrusion frequency of prostheses placed in direct contact with the tympanic membrane led Coyle Shea, as cited by Brackmann et al,[10] to propose cartilage interposition, and this has resulted in a reduction of the extrusion rate by approximately 50% to 70%[9,13] that corresponds well with our experience. Use of either perichondrium alone or cartilage appeared to be equally effective in preventing or delaying extrusion, which so far has occurred in 20% of these procedures. Others recently have reported much lower rates. Silverstein et al[11] found that only 5.5% of prostheses were extruded, but their observation period exceeded 24 months in only 20% of their 104 procedures. Extrusion occurred in 10% of 141 reconstructions performed by Jackson et al,[9] the average duration of follow-up being 20 months with a maximum of 4 years. Brackmann et al[10] had an extrusion frequency of 7%, with two thirds being detected within 2 years of operation; their follow-up period was apparently from 4 months to 4 years.

In the present study, the duration of follow-up ranged from 1 month to 8 years with a mean of 45 months, and was less than 2 years, mostly as a result of reoperation, in 18 of the procedures (26%). Extrusion so far has been observed from 1 to 72 months after operation, with ten of the 27 extrusions (37%) occurring within the first 2 years. This is only one half of the extrusion frequency reported by Brackmann et al[10] at an equally short observation time. Prosthesis extrusion would appear to be a continuing process, with a frequency that is in inverse proportion to the length of the postoperative observation period. This seems to be a recurring pattern in tympanoplasty surgery of severely diseased ears, and it is probably significant that similar interposition of cartilage recently has been recommended for the ceramic implant.[29] It also may be significant that hearing results are only marginally better in ears in which the prostheses have been retained, a result that has also been reported in other studies.[10,13]

The hearing results in this series are disappointingly poor. However, others have reported comparable experiences. Hicks et al[28] achieved a 26.6-dB mean improvement in air conduction threshold 2 months after operation, which fell to 11.5 dB when the same ears were reviewed 6 to 21 months later. Closure of the 0.5- to 2-kHz air-bone gap to within 10 dB has been reported in only 25% of TORP ears at 5 years after operation.[13] This 10-dB criterion has been regarded as too severe, and results have been deemed acceptable when the air-bone gap was as large as 20 to 30 dB.[9,10]

Silverstein et al[11] recently compared their own TORP and PORP results with those from two other series in the literature.[9,10] The average of the 0.5- to 2-kHz air-bone gap was used as a criterion, but only after all surgical failures, not only middle ear problems but also sensorineural hearing losses, were excluded. Assessment of any form of medical treatment should not be restricted to successful cases alone. Frequent surgical failures may well indicate that the problem and the operative solution are incompatible.

The present results for both TORP and PORP procedures are compared in Table 4 with the PORP figures from two series,[9,10] the PORP figures being chosen since they are better than those for TORPs in both studies. Our results are somewhat poorer, especially in the larger air-bone gaps. However, all procedures performed by us, irrespective of final outcome, postoperative infection, prosthesis extrusion, or need for subsequent revision surgery, have been analyzed in our study. Under these circumstances, the present results do not appear so very inferior to those in the cited reports.

Recently, the results obtained with presculpted cartilage TORPs and PORPs have been reported, and good results claimed with a 0.5- to 2-kHz air-bone gap being achieved at the 20-dB level in 64.7% and at 10 dB in 25.5% of cases.[30] Our corresponding figures are 52% and 35%, which is an acceptable approximation. These results do not, however, give any cause for satisfaction. What these percentages of air-bone gap closure mean for the patients' functional status can be better assessed from the postoperative air conduction thresholds (Table 3), with mean values ranging from 40 to 55 dB, and from the fact that approximately one quarter of the patients are now regular hearing aid users.

Reduction of the 0.5- to 2-kHz pure tone average air-bone gap to different levels should not be employed as the sole means of assessing the results of middle ear reconstructive surgery. If pure tone audiometry is employed, hearing gain or loss obtained by comparison of the preoperative and postoperative air conduction thresholds is a more meaningful measure. Emphasis should be placed on the functional outcome and the degree to which patients obtain lasting socially adequate hearing after surgery. This has not been provided in our hands by these synthetic prostheses, which are no longer in use in our department.

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XXI CONGRESS OF THE INTERNATIONAL ASSOCIATION OF LOGOPEDICS AND PHONIATRICS

The XXI Congress of the International Association of Logopedics and Phoniatrics will be held August 6-10, 1989, in Prague. For further information, contact the Secretariat, XXI Congress of the International Association of Logopedics and Phoniatrics, Czechoslovak Medical Society J. E. Purkyne, 120 26 Prague 2, Vitezneho unora 31, Czechoslovakia; telephone 29 41 41-4, telex 121 293.

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FACIAL NERVE ELECTRONEUROGRAPHY WITH IMPLANTED ELECTRODES

EXPERIMENTAL STUDY IN THE RABBIT

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Facial nerve function was evaluated with permanently implanted electrodes for registration of electroneurography in an experimental model. The test-retest reproducibility was analyzed under normal conditions, as well as after facial nerve section. The mean observed intraindividual variation obtained at different sessions was 4%. We believe that the method is well suited for studies of experimental facial palsy.

KEY WORDS — electroneurography, facial palsy, implanted electrodes.

INTRODUCTION

Electroneurography (ENoG) is an electrophysiologic test for evaluation of facial nerve function.[1-3] A supramaximal stimulation of the nerve is believed to depolarize all functioning neurons, and to create a compound muscle action potential (CMAP). This CMAP is usually recorded with a bipolar electrode placed on the skin overlying a specific muscle group.[1,4,5] Shape and size of CMAP recordings will vary significantly depending on the position of the electrodes in relation to the motor points, skin resistance, and electrode pressure against the skin.[2,5-7] Because of these factors, acceptable test-retest reproducibility of CMAPs is often difficult to obtain. In an experimental model, these problems can be reduced to a minimum through a fixed electrode position with implanted electrodes for stimulation and recording.

This experimental design aims to create a standardized condition for repeated recordings of normal nerve conduction, as well as facial nerve function under pathologic conditions.

MATERIALS AND METHODS

Sixteen rabbits of different breeds and both sexes, weighing between 3.5 and 5.0 kg, were used in the study. The experimental design was approved by the local ethics committee. All operative procedures were performed under general anesthesia with ethanyl-fluanison (Hypnorm) 0.3 mL/kg body weight in combination with diazepam (Valium) 5 mg/kg body weight, given intramuscularly. Additional local infiltration of lidocaine (Xylocaine) 10 mg/mL was given at the operative sites. The anesthetic effect lasted approximately 40 minutes, after which an additional dose of ethanyl-fluanison could be given at half the amount of the initial dose. This could be repeated safely several times. When we recorded the CMAPs, the animals had to be anesthesized by a similar procedure, except that lidocaine was omitted this time.

A connector (Cannon DFGS 2000 D) destined to be fixed to the skull of the animal was prepared (Fig 1). A multistranded, Teflon-coated stainless steel wire (Clark Medical 7SS-2T) was used for the

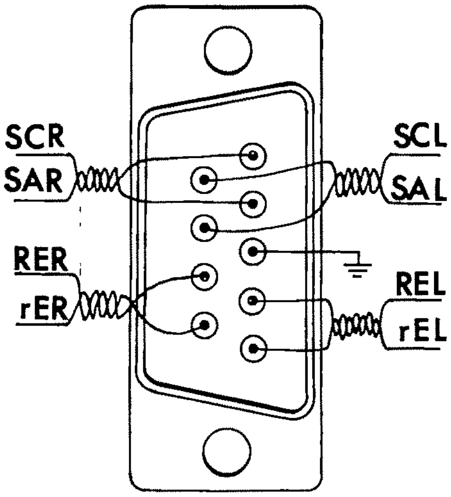

Fig 1. Diagram of connector and its wiring. Stimulation and recording electrodes are twisted around each other to minimize electrical interference and separated as far as possible from each other. SCR — stimulation cathode right, SCL — stimulation cathode left, SAR — stimulation anode right, SAL — stimulation anode left, RER — recording electrode right, REL — recording electrode left, rER — reference electrode right, rEL — reference electrode left.

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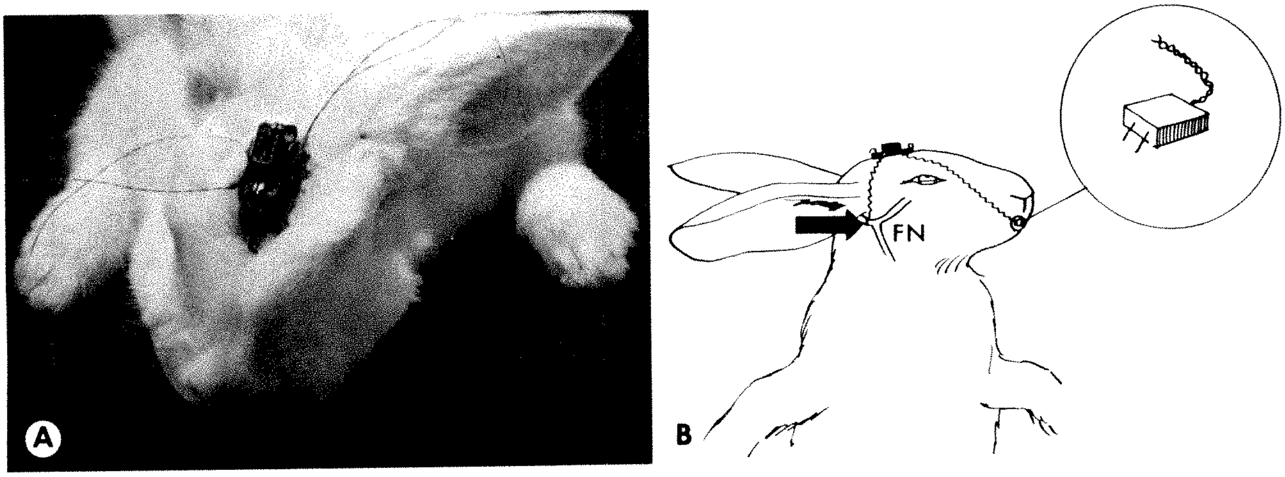

Fig 2. A) Photograph of rabbit with connector in place. B) Schematic drawing of connector and electrodes in place. Stimulation electrode is placed adjacent to stylomastoid foramen (arrow). Recording electrode is positioned in lower lip. Inset) Magnification of electrode tip embedded in composite. FN — facial nerve.

electrodes. The bottom of the connector was isolated with methyl-methacrylate (CMW type 1 orthopedic bone cement) to prevent electrical interference.

Through a midline sagittal skin incision, two holes were drilled in the exposed skull bone, without penetrating into the cerebral cavity. Stainless steel screws were used to secure the connector to the underlying bone (Fig 2A). Skin incisions were performed preauricularly and in the lower lip. The electrodes were passed subcutaneously to their respective positions: the stimulation electrode adjacent to the stylomastoid foramen and the recording electrode in the lower lip (Fig 2B). The Teflon insulation was removed from the wire ends for a length of 5 mm and the wire ends were embedded in an ultraviolet light-cured composite (Aurafil) to prevent electrode displacement (Fig 2B inset). The device, holding either the stimulation or recording electrode, was placed in a subcutaneous pocket and kept in place by a silk suture. After 2 weeks, the electrode device was firmly incorporated in scar tissue.

The skull connector was attached to an electromyography unit (Medelec, M54). The square-wave stimulus intensity could be varied from 0 to 50 mA at a fixed duration of 0.1 ms with a frequency of 1 c/s. The CMAPs were visualized on an oscilloscope screen and documented with a Polaroid camera. In order to obtain good visualization of the recordings, we planned for each photographic exposure to consist of seven consecutive responses. The resultant CMAP had a typical configuration with a stimulation artifact preceding a large negative component followed by a smaller positive component (Fig 3). The stimulus intensity was increased until no further increase in CMAP amplitude was observed. An additional 20% increase of stimulus intensity was added in order to create a supramaximal stimulation.[2]

The CMAP amplitude was measured with a ruler from the Polaroid photos as a peak-to-peak value (Fig 3).[1,4,6-8] Recordings were generally performed once a week, starting 2 weeks after implantation. Daily recordings were performed on two animals, starting 4 weeks after implantation.

In order to evaluate the method under pathologic conditions, we performed a painless facial nerve transection after having obtained stable CMAP recordings on two animals. Through a retroauricular incision the intratemporal facial nerve canal was exposed. The tympanic portion of the nerve was identified and transected, whereupon a 2-mm segment of the nerve was removed. Postoperative recordings of CMAPs were performed until no further electrical activity was identified.

RESULTS

The animals tolerated the implanted electrodes well. The CMAP recordings obtained are summarized and statistically analyzed in Table 1. The

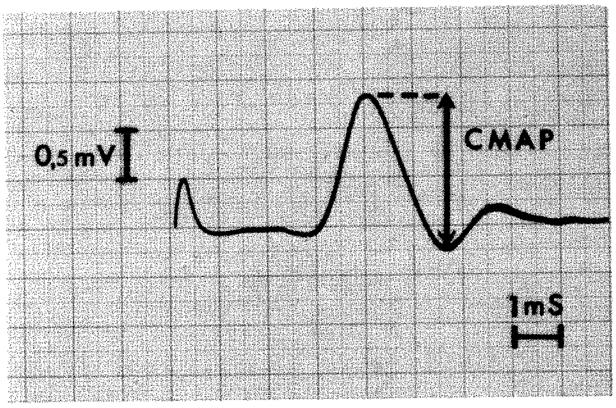

Fig 3. Printout of seven consecutive recordings of nonaveraged compound muscle action potentials (CMAPs) obtained over 7 seconds. All recordings are identical and superimposed on each other. No intraindividual variation was detectable.

TABLE 1. COMPOUND MUSCLE ACTION POTENTIAL

Animal	*No. of Recordings*	*Mean CMAPs (mV)*	*SD*	*Intraindividual Variation (%)*
1	3	0.25	0	0
2	3	0.98	0.029	3
3	8	1.70	0.100	6
4	2	1.70	0	0
5	2	1.90	0	0
6	5	0.30	0.029	10
7	4	0.58	0.055	9
8	3	0.64	0.032	5
9	3	0.57	0.032	6
10	2	1.38	0	0
11	4	1.34	0.082	7
12	4	0.93	0.041	4
13	8	0.74	0.038	5
14	4	0.29	0.012	4

Recordings were made once a week, starting 2 weeks after implantation. In animals 3 and 6, daily recordings were performed starting 4 weeks after implantation. Standard deviation and intraindividual variation were calculated according to established norms.

CMAP — compound muscle action potential.

CMAP remained stable and no detectable variation was seen on any recording occasion. The mean observed intraindividual CMAP variation obtained at different sessions was 4% (Table 1).

On the two animals on which nerve transection was performed, the immediate preoperative and postoperative CMAP recordings were practically identical. The animals developed a complete ipsilateral facial palsy. The CMAPs were recorded at regular intervals postoperatively and the amplitude of the CMAP diminished over time (Table 2). No electrical activity was recorded upon stimulation 42 hours after transection of the nerve.

DISCUSSION

Permanent, implanted electrodes have been used previously for cochleoneural potential recordings from the round window in guinea pigs.[9,10] Laboratory rodents have been shown to tolerate implanted electrodes for several months[9,10]—an observation in accordance with the findings of the present study. Occasionally in our experiments, minor infection occurred around the screws that held the connector in place in the skull bone. This, however, did not cause any disturbance in the function of the electrodes. In spite of the stability of the preparation, the connector, which is firmly attached to the skull bone, invariably will become disconnected with time. Repeated mechanical injuries to the connector site occur as the animal moves around in the cage. Local infection and rejection may contribute to the detachment of the connector. The design, however, remains stable for an adequate period of time for the conclusion of the experiment, ie, >6 weeks.

Prior to embedding the electrodes in composite, electrode displacement was a common source of experimental failure (these animals are not included in the present report). After this modification of the electrode tips, the electrodes were found to be firmly incorporated in scar tissue 2 weeks after the operation, whereupon stable CMAPs were obtained. Variation of the position of the recording electrode will significantly alter CMAP appearance, polarity, and amplitude. In a routine testing situation the placement of the stimulating electrode may vary somewhat but the placement of the recording electrode must be kept constant.[4] In order to obtain supramaximal stimulation without inducing responses from muscle groups innervated by adjacent nerves, the stimulation electrode sometimes was gently manipulated so that this requirement could be achieved.

TABLE 2. REDUCTION IN COMPOUND MUSCLE ACTION POTENTIAL AFTER NERVE TRANSECTION

Postoperative Time Interval (hr)	*CMAP Reduction*	
	Animal 15	*Animal 16*
0	0%	0%
20	40%	52%
24	55%	73%
28	64%	NR
36	85%	NR
42	100%	100%

NR — not recorded, CMAP — compound muscle action potential.

Previous investigators using facial nerve ENoG have reported test-retest variability between 6.2% and 20% in clinical materials.[3,4,6,7] To our knowledge, no similar animal experiments have been performed. It is therefore difficult to compare our experimental results with those of these clinical studies. A mean variation of 4% in a weekly test-retest situation was found in the present material. This proves the stability of the method and should allow more detailed analyses of even small conductivity changes in the facial nerve. The peak-to-peak amplitude of the CMAP is considered to be roughly proportional to the number of muscle fibers that respond to the nerve impulse.[11] This empiric rule, however, has not been substantiated by a controlled experimental study proving this linear relationship.

On the two animals on which nerve transection was performed, the CMAP recorded prior to the experiment was used as a reference value. The cessation of conduction in the distal segment of the nerve after nerve transection occurred as expected and corresponded well with that in other investigations, despite time factor variations in some similar experiments.[12-16] The reduction of the CMAP amplitude was thus progressive with time until no responses could be obtained 42 hours postoperatively.

CONCLUSION

Facial nerve ENoG with implanted electrodes allows repeated measurements of facial nerve conductivity for at least 6 weeks. The fact that investigator bias can be reduced to a minimum is of importance in this kind of experimental work. The method is well suited for studies of experimental facial palsy with a minimum of test-retest variation. Such studies are under way.

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THE DEAFNESS RESEARCH FOUNDATION
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DIMENSIONS OF AND VIBRATIONS IN THE ROUND WINDOW MEMBRANE IN CATS

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The shape and size of the round window membrane and its vibratory pattern were investigated in cats by use of an optical displacement meter and time-averaged holography. The round window membrane is convex toward the scala tympani and appears to be almost elliptic in shape. The major and minor axes of the membrane are 2,273 and 1,934 μm. The mean degree of convexity is 1,975 μm. Under vibration the area of maximum displacement occurs anteriorly-superiorly at low frequency. As frequency is increased, the maximum displaced area shifts posteriorly-superiorly, then breaks up into several segments.

KEY WORDS — optical displacement meter, round window membrane, time-averaged holography.

INTRODUCTION

The round window membrane (RWM) has drawn renewed clinical attention since perilymphatic fistula was recognized as a clinical entity.[1] It already has been noted that the membrane is involved in various ear diseases[2-4] and that via the membrane, therapeutic methods for ear diseases have been facilitated.[5] The structural organization of the RWM in humans and experimental animals has been described in detail.[6-8] Few data, however, have been published on the exact shape and the physical properties of the RWM because of its inaccessible location. This study investigated the shape of the RWM using an optical displacement meter and observed the vibration pattern of the membrane with time-averaged holography. The results and techniques may serve as a basis for new experimental designs in different fields.

METHODS AND MATERIALS

Twelve live cats weighing 2,500 to 3,000 g and 20 fresh cat cadaver specimens were used as materials. The live cats were used for a time-averaged holography experiment, and the cadaver specimens for optical displacement measurement. All surgical procedures were done with use of pentobarbital (30 mg/kg) anesthesia.

Tracheostomy was performed and ventilation was maintained with a respirator. The animals were immobilized with an intramuscular injection of 6 mg/kg tubocurarine.

The RWM was exposed by removing the tympanic bulla through a wide dorsolateral approach. Middle ear components were left intact, and the mucous membrane was cut around the round window niche at a distance of 1 mm by means of a bipolar electric cautery to reduce the fluid seepage from inside the niche.

The fringe of the RWM was removed gently with a diamond bur in order to make the whole membrane visible. The membrane then was coated with a fine bronze powder applied by an airbrush. The animal was attached firmly to the measurement apparatus (Narishige BP-2).

Sound stimuli were delivered through dynamic earphones (TDH-39P). A plastic tube coupled the earphones to hollow stereotaxic earbars. The total length from the tympanic membrane to the earphones was approximately 10 cm. Pure tones over a wide range of frequencies (1 to 20 kHz) were derived from an oscillator. The pure tones were calibrated by a 0.4-mL coupler near the eardrums.

Optical Displacement Meter. The optical displacement meter (Anritsu M533L), as shown in Fig 1, is a device that measures the shape and size of an object without touching it.

A helium-neon laser beam was focused through an illuminating lens and then projected onto the surface of the membrane. Rays that were reflected off the surface and dispersed went through an image-forming lens and were projected onto the position sensor of a light detection element. The element consisted of a thin resistance layer over a Silastic board and had photoelectric properties. The element emitted an electric current at its incidence when light fell on it.

For measuring, the object was placed on a stage coupled to a rotary encoder. The stage was able to move in two directions, forming a right angle at 1-μm intervals, and was adjusted so that the beam would cross the longitudinal or horizontal axis of the membrane. Then the stage was redirected horizontally as shown in Fig 2. The photomicrometer (M533L) measured the longitudinal position of the object, and the rotary encoder generated pulses proportional to the degree of the horizontal move-

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From the Department of Otolaryngology, University of Tokyo. Supported by a Grant-in-Aid for Scientific Research from the Japanese Ministry of Education, Science and Culture (57771037).

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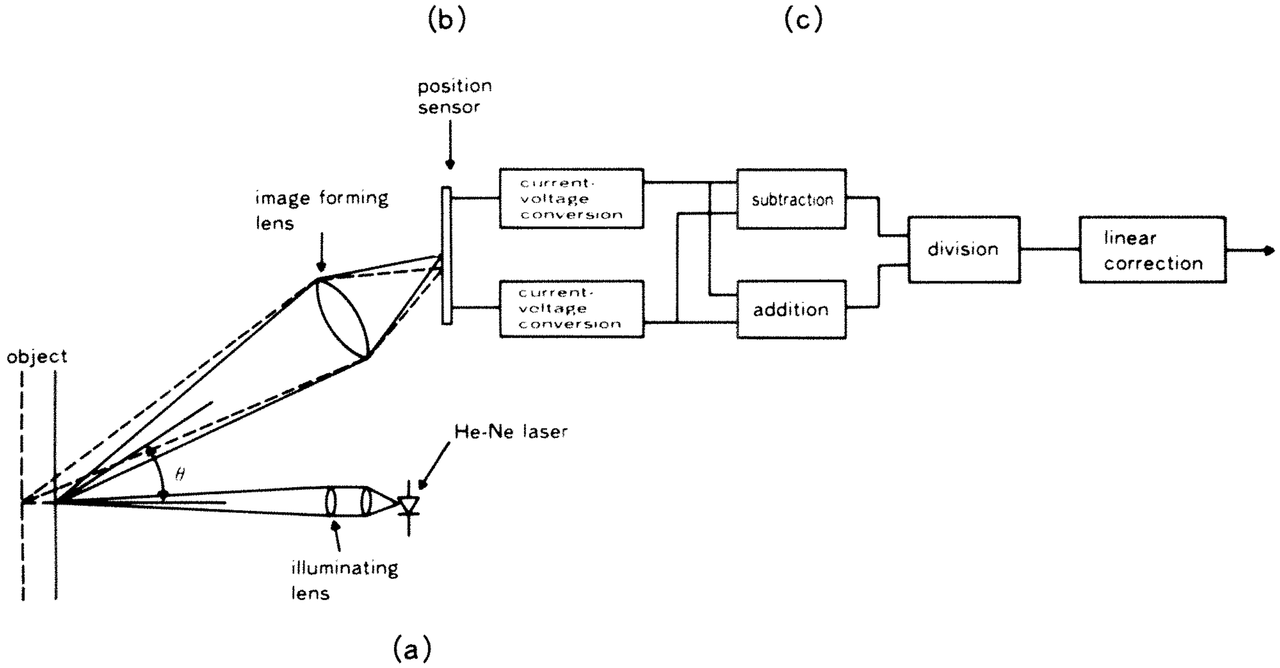

Fig 1. Block diagram of optical displacement meter. Anritsu M533L is composed of helium-neon laser (a), light detection element (b), and amplifier and processor (c).

ment of the stage. These data were stored in a data recorder and processed by a computer (DEC PDP 11/34). The scanning was conducted at 10-μm intervals in both directions under computer control. The results were recorded by a digital plotter.

Time-Averaged Holography. As can be seen in Fig 3, a He-Ne laser (NEC, 25 mW) beam splits in two by means of a beam-splitting half-mirror. The objective beam was projected onto the surface of the RWM, then the reflected rays were focused through a lens onto the holographic plate. The reference beam was passed through the lens and reflected onto the holographic plate to make the real image behind the holographic plate.

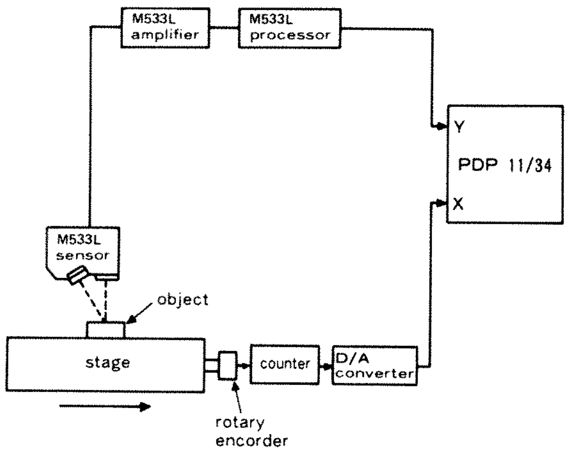

Fig 2. Block diagram of equipment for optical displacement measurement. Object is placed on stage and moved horizontally. M533L sensor measures vertical displacement, and rotary encoder coupled with stage measures horizontal displacement.

In every experiment, a series of holograms was recorded at frequencies from 1 to 20 kHz at various stimulus intensities. Plates were processed and viewed while illuminated by the reference beam. Photographs were taken of all holograms.

RESULTS

Photomicrographic Reconstruction. When the RWM is sectioned in the plane parallel to its superior-inferior axis (Fig 4), it appears to be elliptic in shape, and the membrane is convex toward the scala tympani in every portion. As can be seen in Fig 4, the membrane is rather smooth in its inferior part, whereas its superior part is likely to be rough and steep.

Since it is impossible to trace all specimens in ex-

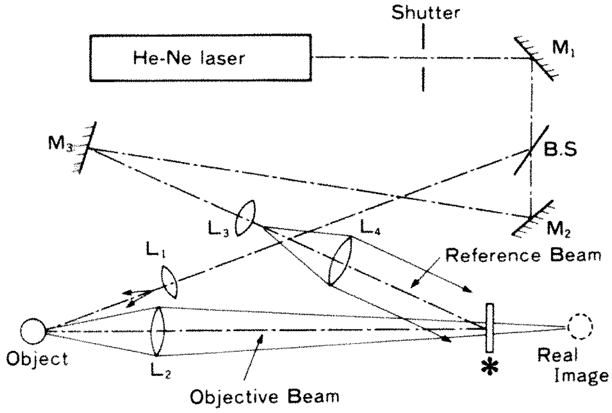

Fig 3. Optical mechanism for holographic recording. Real image is made behind holographic plate (✱). M_1, M_2, M_3 — mirrors, B.S — beam-splitting half-mirror, L_1, L_2, L_3, L_4 — lenses.

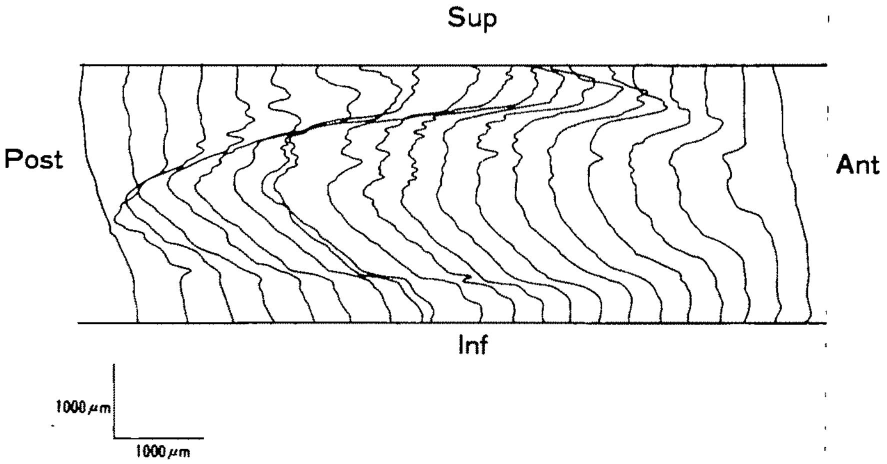

Fig 4. Shape of right round window membrane in plane parallel to its superior-inferior axis. Scanning is conducted at 10-μm intervals on superior-inferior axis. In every trace, leftward deflection shows protrusion toward scala tympani. Vertical scale indicates length along superior-inferior axis. Horizontal scale indicates convexity of membrane.

actly the same plane, each specimen was sectioned at a slightly different angle. The largest diameter and greatest convexity of the RWM in the series were recorded.

The maximum distance of the RWM along its superior-inferior axis varied from 2,058 to 2,498 μm (mean 2,273 μm, SD 138 μm), and it was located in the anterior half of the membrane.

The maximum convexity of the RWM was measured from a line drawn between the superior and inferior edges of the membrane, and it varied from 1,834 to 2,170 μm (mean 1,975 μm, SD 96 μm). It was also located in the anterior half of the membrane.

Figure 5 shows cross sections of the RWM in the plane parallel to its anterior-posterior axis. These records also were made using the largest measurements from each series.

The section of the membrane in this plane was almost elliptic in every section, but it was rather steeper at the bottom than was the membrane in the plane parallel to its superior-inferior axis. The bottom showed a shallow boundary running from the inferior margin to the superior portion. The boundary dividing the membrane into two portions was not straight, but was shaped like the letter C. The maximum distance along its anterior-posterior axis varied from 1,750 to 2,200 μm (mean 1,934 μm, SD 115 μm), and it was located in the inferior third of the membrane. The maximum convexity of the membrane varied from 1,310 to 1,820 μm (mean 1,600 μm, SD 134 μm), and it was located in the superior half of the membrane. A three-dimensional display of the RWM shows a steep surface in its posterior portion and a flat bottom (Fig 6).

Time-Averaged Holography. The time-averaged holographic photographs contain a series of dark and bright fringes. These fringes represent isoamplitude contours. In our present experiments, no fringes were obtained below an amplitude of 2.4 x 10^{-7} m. When the amplitude of the RWM vibration reached 2.4 x 10^{-7} m, it became dark for the first time. With a further increase in amplitude, an area of the RWM became alternately dark and bright. In Fig 7, fringes and corresponding displacement amplitudes are shown. There are five dark and six bright fringes. The displacement amplitudes go up with the fringe number, and the sixth bright area represents the maximum displacement area of the RWM in this particular condition.

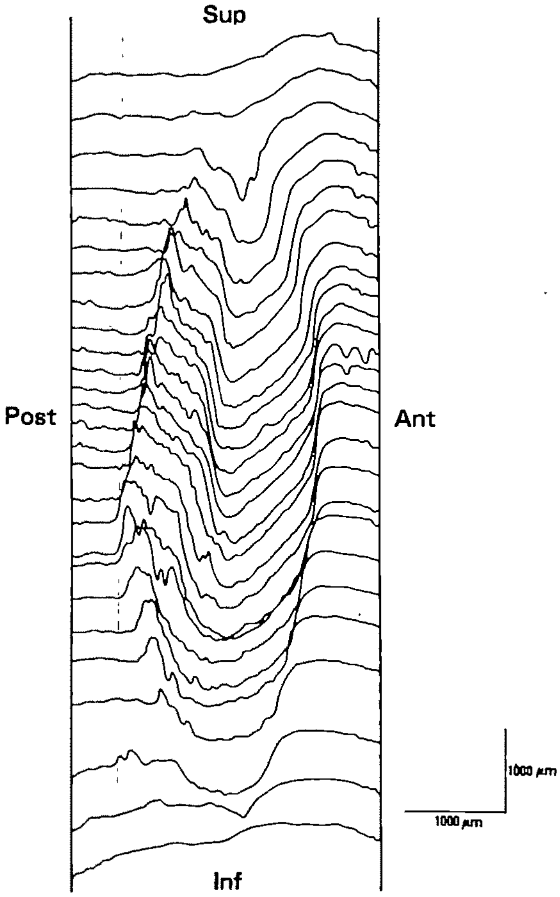

Fig 5. Shape of right round window membrane in plane parallel to its anterior-posterior axis. Membrane scanned is same as in Fig 4. In every trace, downward deflection shows protrusion toward scala tympani. Horizontal scale indicates length along anterior-posterior axis. Vertical scale indicates convexity of membrane.

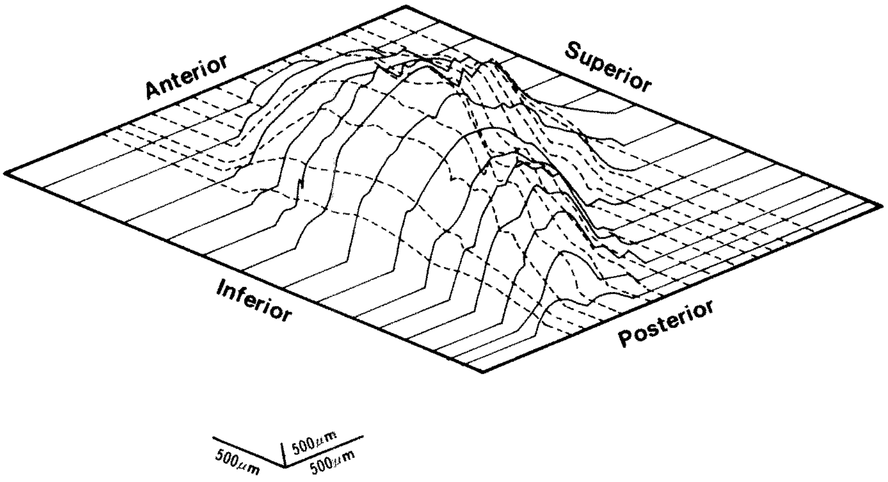

Fig 6. Three-dimensional display of right round window membrane. Top of display is bottom of membrane.

The number of fringes and their spatial arrangement varied considerably with intensity and frequency. As can be seen in Fig 7, the number of fringes increased as stimulus intensity increased. However, there was no clear change in vibratory pattern with stimulus intensity alternation.

Figure 8 shows the vibratory patterns of the RWM at various frequencies. At a low frequency (1,000 Hz), the maximum displacement area invariably was located anteriorly-superiorly. With an increase in frequency, the maximum displacement area was seen to move posteriorly-superiorly. At a high frequency (6,000 Hz), the vibratory pattern broke up into several segments and showed no maximum displacement area.

DISCUSSION

Time-averaged holography was introduced originally by Powell and Stetson.[9] This technique was designed especially for the study of vibrating objects and has been applied in otolaryngology to studies on the tympanic membrane,[10] the ossicular chain,[11] and the RWM.[12] The technique has an acceptable dynamic range even for small objects and is independent of frequency. The disadvantages of the method are the requirements of a wide exteriorization, stability on the part of examined objects, and a reflecting surface. In holographic reconstruction, the relation between the direction of illumination and viewing and the direction of vibration is important. When the object is vibrating at right angles to the direction of illumination and viewing, no movement will be recorded. The error in recording, however, is within 20% for angles as large as 45°.[13]

The optical displacement meter was designed for the study of the size and shape of a small object without directly touching it. The resolution power of this method, however, is less than that of time-averaged holography. The latter obtains the first fringe when the vibration amplitude of the object reaches 2.4×10^{-7} m, while the former can detect displacement only when the displacement amplitude reaches 1.0×10^{-5} m. Thus the optical displacement meter is ideal for the study of the shape and size of a resting object. The combination of these two techniques makes it possible to observe the physical properties of a small object under natural physiologic conditions.

Our results revealed that the RWM is not a flat membrane, but curves toward the scala tympani as a whole. The magnitude of vibration of the RWM varied considerably between animals. But the area of maximum displacement for frequencies below 2 kHz occurred anteriorly-superiorly in all animals. The areas seemed to move posteriorly-superiorly with increased frequency and then broke up into several segments. Consequently, the superior half of the RWM vibrated more easily than did the inferior

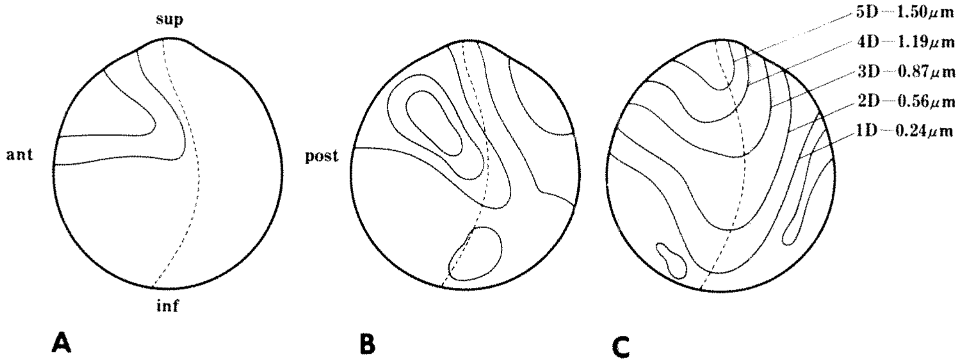

Fig 7. Vibratory pattern of round window membrane as function of stimulus intensity: A) 120 dB, B) 131 dB, C) 140 dB. In C, dark fringes and corresponding displacement amplitudes are noted (1D is first dark fringe, etc). Dotted line shows boundary dividing membrane into two portions.

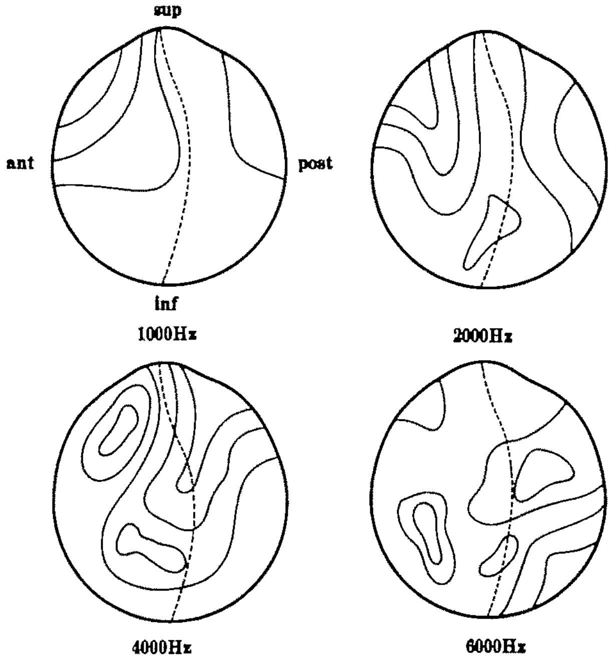

Fig 8. Vibratory pattern of round window membrane as function of stimulus frequency. As frequency is increased, maximum displacement area shifts from anterior-superior to posterior-superior. Stimulus intensity is 136 dB for each plate.

half of the membrane for wide ranges of both frequency and intensity. The results are consistent with those of Khanna and Tonndorf.[12]

No fringe lines were obtained at intensities below 120 dB sound pressure level. The intensities are so great that the RWM may behave nonlinearly; nevertheless, the number of fringe lines increased with sound pressure. The relation between fringe number and displacement amplitude was given by a zero-order Besel function. Thus displacement amplitude increased with sound pressure level in a linear manner. However, holographic fringe lines were wiped out completely when they were taken in live cats subjected to high-intensity sound over long periods (10 to 30 seconds). This phenomenon was never seen to occur with cadaver specimens. Khanna and Tonndorf[12] supposed that the phenomenon was due to perilymphatic pressure (or volume) change during the period of exposure.

It is known that the fibers in the middle layer of the RWM are arranged in a certain direction in humans[14] and experimental animals.[15] In humans, the fibers fan out slightly from the crista semilunaris to the opposing margin of the round window. The same finding has been reported in dogs and guinea pigs. Our vibration experiment suggests that the elasticity of the RWM is not even, but is larger in the superior half, especially near the margin of the RWM, than in the inferior half of the membrane. The relation between the arrangement of the fibers and the vibratory pattern requires further investigation.

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PROGNOSIS OF SECRETORY OTITIS MEDIA IN RELATION TO VISCOELASTICITY OF EFFUSIONS IN CHILDREN

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Both elasticity (G′) and viscosity (η′) of middle ear effusions (MEEs) were measured with an oscillating sphere magnetic rheometer and compared with continuance of fluid retention in 93 ears of 69 children with otitis media with effusion (OME). The ears were divided into four groups according to the viscoelastic properties of MEEs at the first myringotomy. Eighty-four percent of the ears in group 2 were free from effusion within 4 months; the difference from the other groups was statistically significant. These results indicate that the mucociliary clearance function plays an important role in the process of recovery from OME.

KEY WORDS — elasticity, mucociliary transport, otitis media with effusion, prognosis, viscosity.

INTRODUCTION

The clinical course of otitis media with effusion (OME) varies greatly with regard to both duration and degree of severity. Recovery from OME requires resolution of effusions from the middle ear, either through the eustachian tube or by absorption from the middle ear mucosa.[1] The clearance or drainage function of the eustachian tube has been assessed by a variety of methods in the past,[2] and it has been reported that the mucociliary clearance function, one of the drainage functions of the eustachian tube, does not work in affected ears.[3] As many middle ear effusions (MEEs) have suboptimal viscoelasticity for mucociliary clearance,[4] rheologic abnormalities of MEEs may be one of the causes of this mucociliary dysfunction.

The purpose of this study was to clarify the relationship between the viscoelastic properties of MEEs and the duration of fluid retention in the middle ear in children.

MATERIAL AND METHODS

Subjects and Treatment. The OME was diagnosed by clinical symptoms, pneumatic otoscopy, pure tone audiometry, middle ear impedance measurement, and tympanometric patterns. Ninety-three untreated ears of 69 children were found to retain MEEs at the first myringotomy, and all the ears were entered into the study. The age distribution of the patients ranged from 3 to 15 years and the mean age was 6.5 ± 2.4 years. The length of history of otitis media ranged from 2 months up to 2 years on inquiry, but it was confirmed that the patients had never received any surgical treatment, such as myringotomy or ventilation tube insertion, in the other clinics. In the present study, the patients were treated with myringotomy under local anesthesia every 1 or 2 months. Adenoidectomy, ventilation tube insertion, and long-term administration of antibiotics, mucolytic agents, or steroids were not done during the study.

Tympanometry. The children who underwent myringotomy were followed up for at least a year by serial tympanometry every 1 or 2 months. All the tympanometric measurements were performed with a Teledyne impedance audiometer (model TA-2C). Tympanograms were classified on the basis of a modification of the Jerger system[5] into four types: type A, middle ear pressure from 200 to −99 mm H_2O; type C_1, from −100 to −199 mm H_2O; type C_2, from −200 to −400 mm H_2O; and type B, a flat curve without a well-defined compliance maximum. All the ears showed B type at the first myringotomy. The number of months required for B type to change into A or C_1 type was used for the criterion of prognosis as the continuance of fluid retention.

Collection of MEEs. At the first myringotomy, MEEs were aspirated with the Juhn Tym-Tap. Those with very high viscosity were aspirated by our making the myringotomy incisions larger than usual. In this way, MEEs were collected from all 93 ears. They were stored at 4°C without any extraction or dilution and prepared for rheologic measurement within 24 hours after collection.

Measurement of Viscoelasticity. Measurements were made with the oscillating sphere magnetic rheometer, which can provide data on viscoelasticity of small sample volumes over a wide range of angular frequency. Details of this instrument are described elsewhere.[6,7] A small amount of test material (about 4 μL) was placed in a disposable cell. An iron sphere 200 μm in diameter was placed within

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RELATION BETWEEN CONTINUOUS DURATION OF FLUID RETENTION AND ELASTIC MODULUS OR DYNAMIC VISCOSITY OF 93 MIDDLE EAR EFFUSIONS

	Duration of Retention	
	<4 mo (No. of Ears)	*≥4 mo (No. of Ears)*
Elastic modulus (G')*		
Group 1 ($G' < 6.31$)	13	13
Group 2 ($6.31 \leq G' < 63.1$)	16	3
Group 3 ($63.1 \leq G' < 1{,}000$)	19	15
Group 4 ($1{,}000 \leq G'$)	5	9
Dynamic viscosity (η')†		
Group 1 ($\eta' < 0.631$)	11	8
Group 2 ($0.631 \leq \eta' < 6.31$)	20	8
Group 3 ($6.31 \leq \eta' < 100$)	17	16
Group 4 ($100 \leq \eta'$)	5	8

*In dyne/cm². Group 1 *v* group 2, $p<.05$; group 2 *v* group 3, $p<.05$; group 2 *v* group 4, $p<.01$.

†In poise. Group 2 *v* group 4, $p<.05$.

the sample and oscillated in a vertical direction by application of a sinusoidally varying magnetic field gradient. The motion of the iron sphere was followed through a light microscope and transduced to an electric signal with an optoelectronic tracer. The amplitude of vibration and the phase lag of the iron sphere with respect to the oscillatory driving force were used to compute the elastic modulus (G') and dynamic viscosity (η') at any given frequency. All measurements were made at 25°C.

Statistical Analysis. The χ^2 test (nonparametric method) was used to compare the prognosis of the disease between groups.

RESULTS

Viscoelasticity of MEEs. The G' at 1 Hz in 93 freshly collected effusions ranged from 1.92 to 5,890 dyne/cm². The η' at 1 Hz ranged from 0.156 to 460 poise. The distribution of both G' and η' of MEEs was divided into four groups (1, 2, 3, and 4) in relation to mucociliary transport. As effective mucociliary transport is obtained at a G' of about 20 dyne/cm² and an η' of about 2 poise in vitro,[4] the G' of group 2 was determined as ranging from 6.31 to 63.1 dyne/cm². This range is equal to 20 dyne/cm² ± 0.5 log units, where the mucociliary transport works best. The G' of group 1 was less than 6.31 dyne/cm², at which effusions appear serous. Effusions with a G' of more than 63.1 were further divided into groups 3 and 4. The G' of group 3 ranged from 63.1 to 1,000 dyne/cm². The G' of group 4 was more than 1,000 dyne/cm², at which effusions appear gluey. The distribution of η' was similarly divided into four groups. The η' of group 1 was less than 0.631 poise, and the η' of group 2 ranged from 0.631 to 6.31 poise (2 poise ± 0.5 log units). The η' of group 3 ranged from 6.31 to 100 poise, and the η' of group 4 was more than 100 poise.

Prognosis of OME. In this study, the prognosis of the disease was evaluated by the number of months required for type B tympanograms to change to type A or type C1 (duration of fluid retention). All the ears were normalized on tympanometric measurements within the study period. The minimum duration was 1 month and the maximum was 36 months. Fifty-seven percent of all the ears had type B for less than 4 months, and 73.1% of all the ears had type B for less than 12 months. In this study, a good prognosis for the disease was arbitrarily defined as fluid retention for less than 4 months. A poor prognosis was defined as fluid retention for 4 months or more.

Viscoelasticity and Prognosis. The Table shows the relation between the prognosis of the disease (duration of fluid retention) and the viscoelasticity of MEEs.

As regards elasticity versus duration of fluid retention, the distribution of good prognoses (fluid retention <4 months) is significantly higher in group 2 than group 1 ($p<.05$), group 3 ($p<.05$), and group 4 ($p<.01$), respectively (Fig 1).

As regards viscosity versus duration of fluid retention, a statistical difference in good prognosis was observed between group 2 and group 4 ($p<.05$). But the number of good prognoses in group 2 was not significant compared with that in group 1 or group 3 (Fig 2).

DISCUSSION

Recovery from OME requires disappearance of the fluid from the middle ear either through the eustachian tube or by absorption from the middle ear mucosa. The middle ear mucosa is reported to be capable of absorbing the watery component.[8] Thus, the viscous component may be cleared through the eustachian tube. Two different mechanisms are involved in clearance through the eustachian tube, namely, muscular clearance and mucociliary clearance. Although muscular clearance can expel more fluid than can mucociliary clearance experimentally,[9] muscular drainage is unlikely to occur in the presence of negative pressure of the middle ear.[10] Therefore, the mucociliary function may play a major role in the clearance of MEE under negative pressure.

Among the factors affecting mucociliary clearance, viscoelastic properties of the mucus, especially elasticity, are an important factor.[11] The mucus in the airway is transported best when its viscoelasticity is at an optimal value, and the efficiency of transport decreases if the viscoelasticity is beyond this value.[12] Using fresh human MEEs, Majima et al[4] reported that maximum mucociliary transport on the mucus-depleted frog palate was obtained at a G' of about 20 dyne/cm² and at an η' of about 2 poise. Below the optimal values, the transport decreases with the decrease of G' and η'. Above the

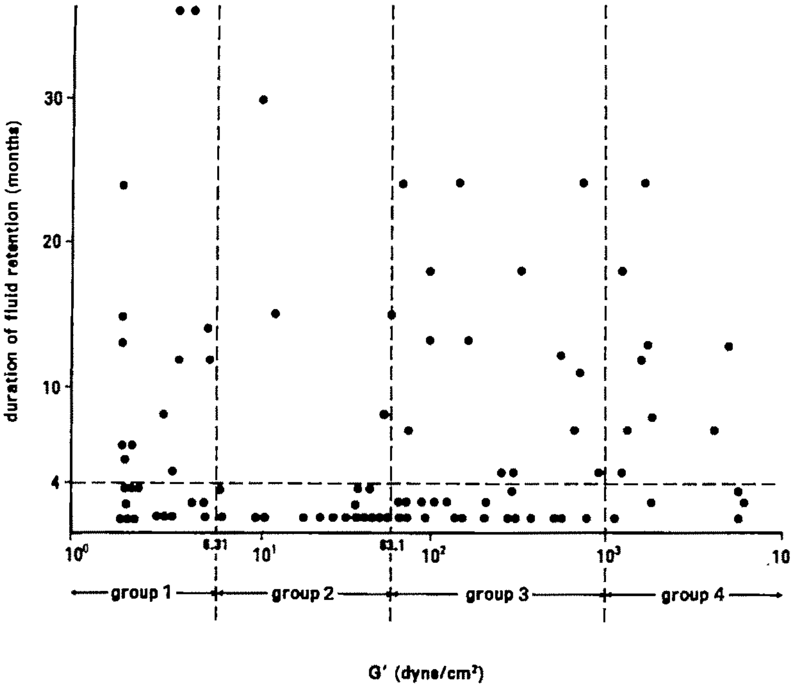

Fig 1. Continuous duration of fluid retention versus elastic modulus G' at 1 Hz. Resolution of fluid in group 2 is faster than in group 1 ($p<.05$), group 3 ($p<.05$), or group 4 ($p<.01$).

optimal values, the transport decreases with the increase of G' and η'. In the present study, the viscoelasticity of MEEs was classified into four categories (groups 1, 2, 3, and 4). The values of G' and η' of group 2 are in the optimal range for mucociliary transport, those of group 1 are too low to produce effective mucociliary transport, and those of groups 3 and 4 are too high.

In this study, the ears with type A and C1 tympanograms are regarded as absent of MEEs. This is because A and C1 tympanograms predict the absence of MEEs with 88% sensitivity and 91% specificity and, correspondingly, B and C2 tympanograms predict the presence of MEEs with 91% sensitivity and 88% specificity.[13]

The relation between the physical properties of MEEs and prognosis of OME rarely has been described. Fiellau-Nikolajsen[13] reported that the viscosity of the MEE found at myringotomy gave no information about the severity of the condition, comparing the gross appearance of MEE and the continuous duration of type B. His criterion on viscosity is merely the macroscopic appearance and thus lacks in preciseness. In this sense, this report is the first to investigate the relation between viscoelastic properties of MEEs and the prognosis of

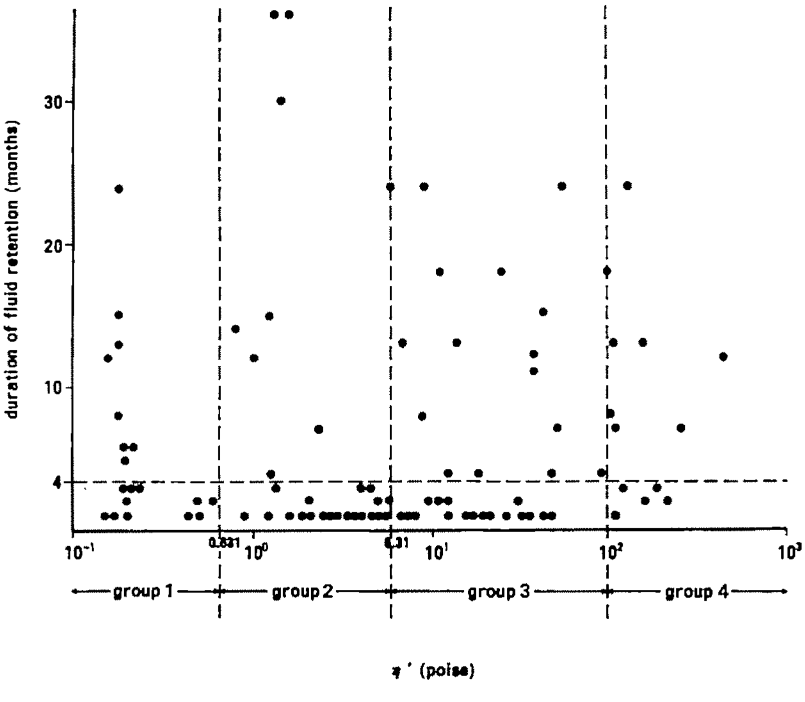

Fig 2. Continuous duration of fluid retention versus dynamic viscosity η' at 1 Hz. Resolution of fluid in group 2 is faster than in group 4 ($p<.05$).

OME.

In the present study, 84% of the ears with MEEs of which the G′ values belonged to group 2 were free from effusion within 4 months. The incidence of the good prognosis (duration of fluid retention < 4 months) was significantly higher in group 2 than in the other three groups. One way to explain the better prognosis of group 2 is that such MEEs are effectively transported by cilia of the eustachian tube and middle ear, and hence are easily cleared from the middle ear. Another explanation is that the pathologic condition of the middle ear in group 2 might be mild and that the secreted mucus might be rheologically normal.

On the other hand, the ears with high viscoelasticity showed a poor prognosis. From the viewpoint of viscoelasticity, the mucokinetic drugs may improve the prognosis of these ears.[14] Roydhouse[15] reported the therapeutic value of bromhexine hydrochloride in OME. Such mucokinetic agents should be administered only to ears with effusions of high viscoelasticity. In this sense, the rheologic measurement of MEEs is important for the choice of treatment.

The results of the present study lead to the following conclusions: 1) the mucociliary clearance function plays an important role in the process of recovery from OME and 2) rheologic measurement of MEE is important for the choice of therapy.

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ANTIGEN-SPECIFIC IMMUNE RESPONSE IN THE INNER EAR

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The specificity of inner ear immune responses was investigated by challenging each inner ear of presensitized animals with different antigens. Animals presensitized systemically with keyhole limpet hemocyanin (KLH) and bovine serum albumin (BSA) were challenged with KLH in the right and BSA in the left inner ears. Two weeks later perilymph anti-KLH levels were increased significantly in the right inner ears compared to the levels in the left inner ears. In contrast, perilymph anti-BSA levels were increased significantly in the left inner ears compared to the levels in the right inner ears. These results suggested that the rise in perilymph antibody following inner ear antigen challenge was predominantly the result of an antigen-specific immune response in the inner ear and not simply the result of an increase in vascular permeability or serum contamination from the experimental procedure itself.

KEY WORDS — bovine serum albumin, guinea pig, inner ear immunity, keyhole limpet hemocyanin.

INTRODUCTION

The concept that the inner ear comes under the influence of an immune system emanates from studies that have shown that upon inner ear antigen challenge 1) a rise in perilymph antibody develops,[1,2] 2) characteristic histopathologic changes occur within the inner ear,[3,4] 3) specific immunity is conferred upon the host,[5] 4) a mounting inner ear immune reaction results in significant cochlear physiologic changes,[3] and 5) the local immune response protects the inner ear from pathogenic organisms.[6,7]

We examined the specificity of the inner ear immune response by employing simultaneous challenge of different antigens into each inner ear. By virtue of this experimental paradigm, we were able to determine if antibody elevations were restricted to the specific antigen inoculated, if they were restricted to the side of antigen challenge, and whether they were the result of increased vascular permeability or serum contamination from the experimental procedure itself.[8,9]

MATERIALS AND METHODS

Animal. Ten female, strain 13 guinea pigs (350 to 400 g) were employed for this experiment (Crest Laboratories).

Antigen. Keyhole limpet hemocyanin (KLH) was obtained from Pacific Biomarine Supply Co (Venice, Calif) and was used in its associated form in complete Freund's adjuvant (CFA) for intradermal immunization. Keyhole limpet hemocyanin was diluted in phosphate-buffered saline (PBS) pH 6.4 for inner ear challenge. Bovine serum albumin (BSA) in CFA was used for intradermal immunization simultaneously with KLH intradermal immunization, and BSA was diluted in PBS pH 7.2 for inner ear challenge.

Antibody Assay. Antibody levels to KLH and BSA were determined by ELISA; the details of this assay have been described previously.[1,2,4] By use of absorbance readings, a binding ratio is calculated for each sample dilution, and a sample is defined as positive at a particular dilution 1) if the binding ratio is >2 and 2) if the mean absorbance of wells containing antigen minus the mean absorbance of wells without antigen is >0.05. A standard curve is constructed by running a known serum with high anti-BSA or anti-KLH titers for each experimental group, and the antibody determinations are expressed in units per milliliter.

Immunohistochemical Assay for Detection of Antibody-Producing Cells. Animals were perfused with warm saline followed by 10% formalin with acetic acid (pH 2.2). The following day the cochleas were dissected and decalcified in 4% EDTA at 4°C. The tissue was embedded in Surgipath (Surgipath Medical Instruments, International, Graylake, Ill), and 4- to 6-μm sections were cut and mounted on chrome alum subbed slides. Air-dried sections were deparaffinized in histoclear, incubated in methanol with 0.06% H_2O_2 to reduce the endogenous peroxidase, and rehydrated in PBS. They then were incubated in 10% normal goat serum to reduce the background staining, incubated with biotinylated, goat anti guinea pig IgG-Fc fragment for 24 hours at 4°C, and reacted with streptavidin–horseradish peroxidase followed by 3-amino 9-ethylcarbazole,

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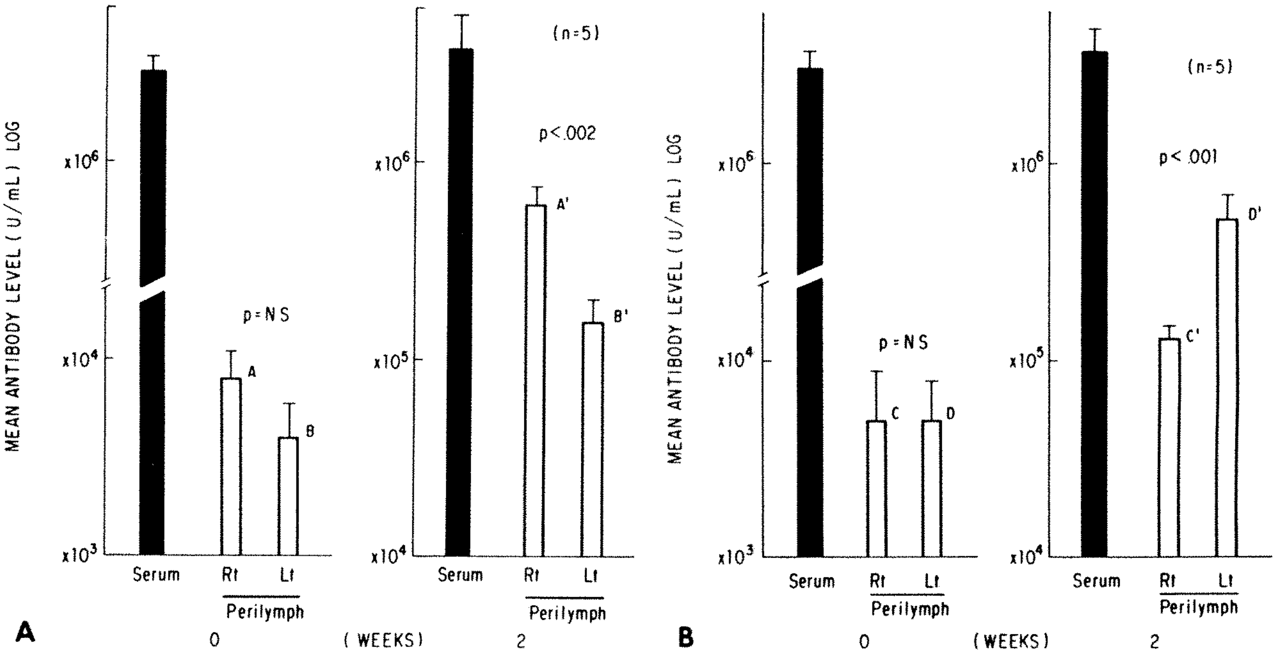

Fig 1. Mean serum and perilymph antibody levels following right inner ear KLH challenge and left inner ear BSA challenge in animals presensitized systemically to KLH and BSA (secondary response). A) Anti-KLH levels. A′ > B′ = $p < .002$ (paired *t* test). B) Anti-BSA levels. D′ > C′ = $p < .001$ (paired *t* test).

the chromagen, and H_2O_2. Representative serial sections were examined with biotinylated goat IgG as a reagent control. Lymph node sections from sensitized animals served as a positive control for plasma cells.

Experimental Design. Animals were immunized systemically by intradermal inoculations with both 500 μg KLH/CFA and 500 μg BSA/CFA, and the inoculations were boosted until high circulating anti-KLH and anti-BSA levels were detectable by ELISA. Clear, grossly blood-free perilymph then was sampled through a microhole hand-drilled into the scala tympani of the basal turn, and the right inner ears were slowly perfused with 250 μg KLH antigen and the left inner ears with 250 μg BSA antigen, each through a micropipet attached to a 500 x 10-μL Hamilton syringe with a repeating dispenser (American Scientific Products, Irvine, Calif).

Two weeks after inner ear antigen challenge, serum and bilateral perilymph samples again were obtained for antibody determinations. The results were expressed as mean antibody levels (±SD). Paired *t* tests and Mann-Whitney U tests were employed for statistical analysis. All animals were perfused and prepared for immunohistochemistry and staining with hematoxylin and eosin.

RESULTS

On day 0, animals showed comparable levels of serum antibody to both KLH and BSA. Mean levels of perilymph antibody to both KLH and BSA measured on day 0 showed no difference between those in the right and left ears (Fig 1). Two weeks after KLH challenge to the right inner ears and BSA challenge to the left inner ears, mean perilymph anti-KLH levels were increased significantly in the right ears compared to those in the left ears (Fig 1A); (right ears [n = 5], $0.610 \pm 0.155 \times 10^6$ U/mL; left ears [n = 5]. $0.156 \pm 0.056 \times 10^6$ U/mL; $p < .002$). On the other hand, mean perilymph anti-BSA levels were increased significantly in the left ears compared to those in the right ears (Fig 1B) (left ears

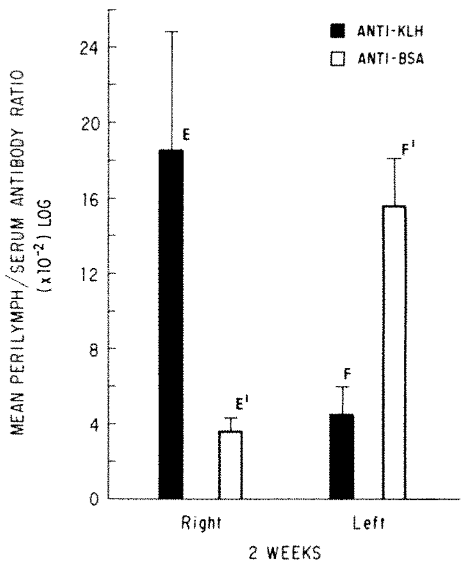

Fig 2. Mean perilymph-serum antibody ratios for anti-KLH and anti-BSA following right inner ear KLH challenge and left inner ear BSA challenge in animals presensitized systemically to KLH and BSA. E > E′ = $p < .001$. F > F′ = $p < .008$ (Mann-Whitney U test).

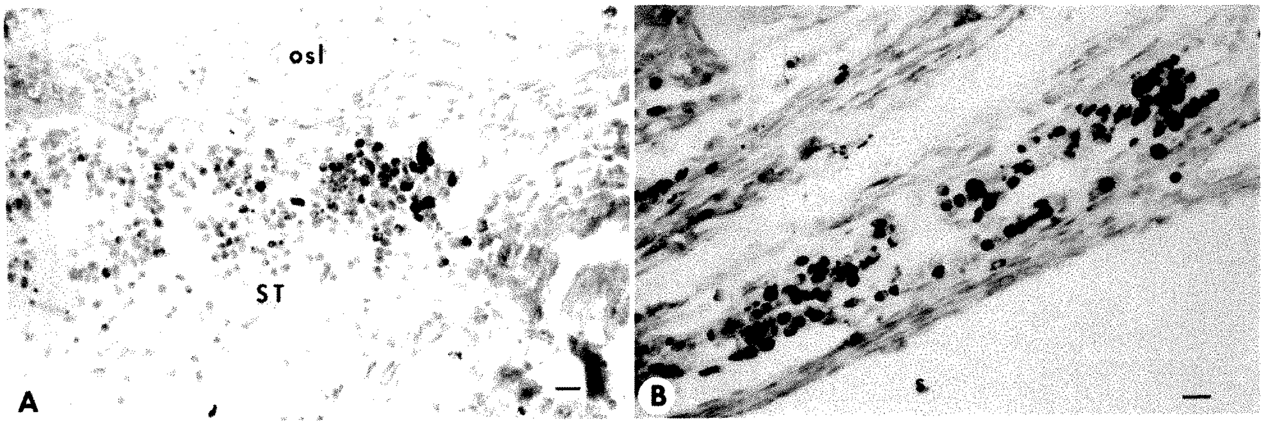

Fig 3. Paraffin sections (6 μm) of temporal bone from sensitized animals challenged with KLH/CFA in scala tympani (scale bar, 30 μm). Plasma cells are labeled immunohistochemically with antibodies to IgG-Fc fragment. Identified are A) scala tympani (ST) just below osseous spiral lamina (osl), and B) perisaccular tissue of endolymphatic sac. s — sigmoid sinus.

[n = 5] 0.524 ± 0.071 x 10^6 U/mL; right ears [n = 5], 0.130 ± 0.022 x 10^6 U/mL; $p < .001$).

The perilymph to serum (P/S) antibody ratio at 2 weeks post–inner ear antigen challenge was significantly elevated only for the antigen inoculated into that side. The P/S antibody ratio for KLH was 18.6 x 10^{-2}, whereas the P/S antibody ratio for BSA was 3.5 x 10^{-2} on the right, KLH-challenged side ($p < .001$, Mann-Whitney U test). Alternatively, on the left, BSA-challenged side, the P/S antibody ratio for KLH was 4.6 x 10^{-2}, whereas the P/S antibody ratio for BSA was 15.5 x 10^{-2} ($p < .008$, Mann-Whitney U test) (Fig 2). These results indicate that the rise in inner ear antibody was antigen-specific and did not result from an increase in vascular permeability or serum contamination of the samples.

Immunohistochemical staining of temporal bones from these animals demonstrated numerous cells stained with anti IgG-Fc. Labeled cells were seen in the perisaccular tissue, endolymphatic sac, perilymphatic space, and perivascular space in the modiolus (Fig 3). Many of the infiltrating cells, therefore, are either plasma cells or B cells. Concurrent identification of these cells as anti–KLH- or anti BSA-producing plasma cells was not performed by immunohistochemical means.

DISCUSSION

In the present study, by injecting different antigens into each inner ear, we had an ideal opportunity to examine whether the evolving rise in perilymph antibody level was specific for the inoculated antigen or was the result of the generalized host response in the serum. Furthermore, there may be communication between the inner ears via the CSF or by an as yet unrecognized homing of sensitized B cells, as is known to occur for mucosal sites, and this experimental paradigm offered the opportunity of examining this possibility as well.

The results from this study clearly demonstrated that the rise in anti-KLH and anti-BSA perilymph levels was restricted to the ear receiving the specific inoculated antigen; that is, the perilymph anti-KLH was elevated in the side inoculated with KLH, and the same for BSA. Examination of the P/S antibody ratios confirmed that the elevation in perilymph antibody for KLH in the right ear and BSA in the left ear was due to local inner ear antibody production and was not due to an increase in vascular permeability or serum contamination of the perilymph samples. Therefore, while filtration through the blood-labyrinth barrier may contribute to perilymph antibody, it cannot be solely responsible for the observed increase in antibody following inner ear antigen challenge.[9]

These results represent further support of our previous hypothesis that the inner ear indeed does possess an immune system that can result in the local production of antibody.[1,2,4] Increasing evidence now suggests that the endolymphatic sac is an important site of antigen processing following antigen challenge of the inner ear, and its ablation severely suppresses the resultant inner ear immune response.[4] The observation of plasma cells and B cells in the perisaccular connective tissue stained with anti IgG-Fc antibody further supports the notion that this site is immunologically active.

If homing of sensitized lymphocytes from the inoculated side to the opposite inner ear had occurred, one would expect that similar antigen-specific antibody levels would have developed on the opposite side, making the observed responses indistinguishable for BSA and KLH. Since this was not the case, it does not appear that homing of antigen-stimulated lymphocytes occurs to any appreciable degree. In recent studies we have found that the earliest immune reaction within a sensitized inner ear consists of the accumulation of many inflammatory cells (lymphocytes and polymorphonuclear leukocytes) around the posterior spiral modiolar veins and perisaccular venules. This suggests that cells leave the systemic circulation and reach the inner ear via these routes. The role of the endolymphatic sac in this response is at present unclear and may involve only antigen processing and an additional site of antibody-producing lymphocytes. The increase in the number of cells in the perisaccular and periductal connective tissue also may contribute to the cellular accumulation seen within the perilymphatic compartment, since these spaces are in continuity. Additionally, the cells seen within the endolymphatic sac and duct, while fewer in number than those seen in the aforementioned sites, may contribute to the cells seen in the scala media in some animals. Whether further expansion of cell lines or specific factors is involved in this proliferation of cells within the inner ear is being investigated currently.

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Ann Otol Rhinol Laryngol 98:1989

MASTOID PNEUMATIZATION IN OTOSCLEROSIS

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The extent of mastoid pneumatization in 150 otosclerotic ears was compared with that of 150 healthy control ears. The size of mastoid pneumatization was measured by use of the Schüller lateral x-ray projection with the help of computed planimetry. The measurements showed the average pneumatized area in otosclerotic ears to be 17.4 ± 5 cm^2, in contrast to 12.9 ± 4 cm^2 for the healthy control ears. The difference between the two groups was highly significant ($p < .0001$). While both groups showed a bell-shaped distribution of the measured pneumatized area, the curve of the otosclerotic ears was shifted significantly to the right. Our findings indicate a link between otosclerosis on the one hand and highly pneumatized mastoids on the other. This link between a hereditary disease and a specific type of pneumatization points to the likelihood that heredity also plays some role in determining the final type of pneumatization.

KEY WORDS — mastoid, otosclerosis, pneumatization.

INTRODUCTION

We studied the size of mastoid pneumatization of patients with otosclerosis because we believed that these patients have an exceptionally highly developed pneumatic system. A search of the medical literature revealed only two references related to this particular question,[1,2] both intimating that patients with otosclerosis have "normal" pneumatization. However, no measurements have been given.

PATIENTS AND METHODS

Two groups of adult patients having the same age-sex distribution were examined.

Group 1. Group 1 consisted of 150 randomized ears of 75 healthy volunteers without any history of ear disease. All patients presented otoscopically normal eardrums and tested as audiologically normal according to their age. Their ages ranged between 18 and 70 years.

Group 2. Group 2 consisted of 150 ears of 147 patients who previously had undergone a stapedectomy when typical otosclerotic stapes fixation had been verified. Preoperative otoscopy revealed intact normal eardrums in all these patients. Their ages also ranged between 18 and 70 years.

Radiographs in the Schüller lateral projection of the mastoid were obtained for all ears in both groups. The limits of the mastoid air cell system were marked on the radiographs (Fig 1). These demarcations were copied on transparent paper and the area (in square centimeters) was measured directly by computed planimetry. A Hewlett Packard 9111-A digitizer operating in conjunction with a Hewlett Packard 9817-H computer was used to assess the area confined to the planimetric measurement. The physician who performed the measurements of the pneumatized area had no information as to whether he was measuring a control or an otosclerotic specimen. All mastoid air cell areas were copied and marked by two independent observers. The differences between the two sets of measurements amounted to 0.2%, which is considered negligible.

RESULTS

Our results are seen in Fig 2. Figure 2A is the histogram of the cross-sectional areas of the 150 mastoid air cell systems of group 1. The mean ± SD cross-sectional area of the mastoid air cell systems in group 1 was 12.9 ± 4 cm^2, with values ranging between 0.8 and 28.9 cm^2.

Figure 2B is the histogram of the 150 mastoid air cell systems of group 2. The mean ± SD cross-sectional area of the mastoid air cell systems in group 2 was 17.4 ± 5 cm^2, with values ranging between 7.1 and 32 cm^2.

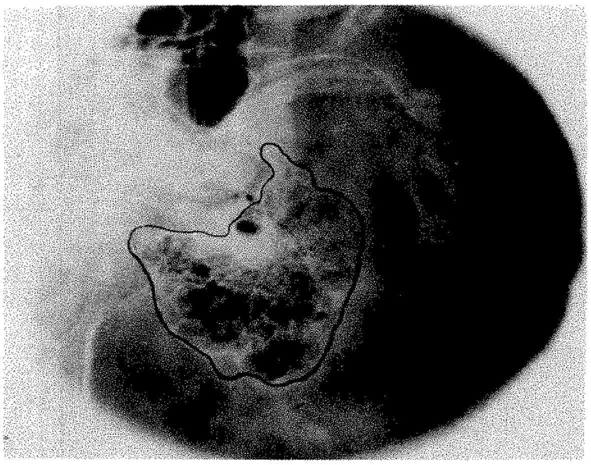

Fig 1. Radiograph of mastoid taken in Schüller lateral projection. Limits of mastoid air cell system are marked.

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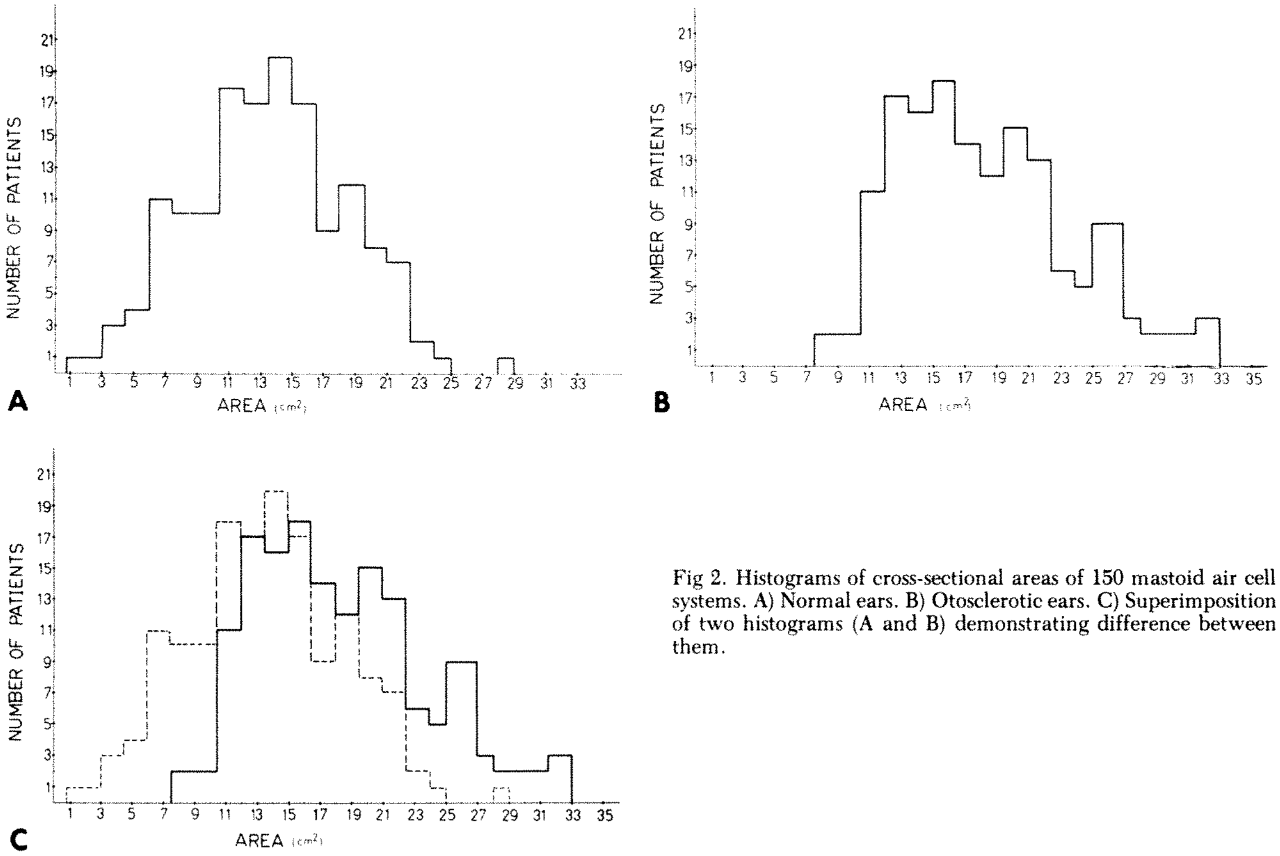

Fig 2. Histograms of cross-sectional areas of 150 mastoid air cell systems. A) Normal ears. B) Otosclerotic ears. C) Superimposition of two histograms (A and B) demonstrating difference between them.

Figure 2C presents the superimposition of the two previous curves, demonstrating the difference between them. The distribution of group 1 is gaussian in type, while that of group 2 shows a shift of the bell-shaped curve to the right. The difference between the means of the two groups is 4.5 cm² or 35% and is highly significant ($p < .0001$). This was calculated by use of the *t* test ($t = 7.549$).

DISCUSSION

Studies that measure the size of mastoid pneumatization usually use a planimetric method.[3-10]

Direct or "total" determination of volume is an impractical method and cannot be conducted on living persons. A good correlation was found between the size of the mastoid system as measured by planimetry and that found by volumetric methods based on Boyles's law.[11] The most representative projection for this purpose was found by Diamant[3] to be the lateral projection of the mastoid, which also was used later by Sadé and Hadas,[6] Tos et al,[7-9] Hug and Pfaltz,[10] and Palva and Palva.[12] One could arrive closer to a realistic absolute value of the mastoid volumes by using figures of correlation and equation of regression for the measured mastoid area. The relative information obtained by the planimetric method also provides us for all practical purposes with the necessary information.

A question in point is to what extent different investigators demarcate the air cell system in a uniform way. In our case we have found a negligible difference between the results of the two independent investigators. Our planimetric studies also show an excellent agreement with Diamant's measurements of a normal cohort.[3] Our mean cross-sectional area for normal ears is 12.9 cm², compared to his 12.7 cm².

Reports concerning pneumatization in otosclerosis are surprisingly scanty. Wittmack[1] had claimed that patients with otosclerosis possess a normally pneumatic mastoid, a notion supported recently by Petrovic and Stanisavljevic.[2] However, these reports do not provide measurements to back up their statements or impressions.

Our study shows that in otosclerotic ears pneumatization is larger and more developed than in normal ears. The statistical analysis showed this trend to be highly significant ($p < .0001$).

While the gaussian curve characteristic of the normal group has some similarities—as far as its shape is concerned—with the curve plotted for the otosclerotic group, important differences in size between the two groups do exist, as the otosclerosis curve shows a significant shift to the right.

The smallest measured cross-sectional area in otosclerotic ears was found by us to be 7.1 cm², whereas the minimal area in the normal ears was 0.8 cm². This observation brings to near zero

($p = .0113$) the probability of otosclerosis occurring in an ear with a pneumatized area of less than 7 cm^2. Furthermore, highly pneumatized mastoids above 25 cm^2 are almost exclusively found in patients with otosclerosis; 20% of the otosclerotic ears showed an area of 25 cm^2 and higher, compared to only 0.66% (one case) of the normal control ears. Therefore, the probability of a normal ear having a pneumatized area of 26 cm^2 or higher is very low ($p = .004$).

Otosclerosis is an autosomal dominant genetic trait that has a low penetrance.[13] Otosclerosis is also indisputably free of environmental factors.

In our study the patients' age range (18 years to 70 years) is far beyond the critical age at which development of the mastoid air cells takes place[3,14,15] and excludes any further possible environmental influence. This study has shown a link between the presence of otosclerosis and the degree of pneumatization and suggests that the expression of the mutant otosclerotic gene is linked to the degree of pneumatization. When a condition whose genetic character is determined, as is the case for otosclerosis, is found to be linked to an anatomic feature, such as mastoid pneumatization, the conclusion that emerges is that this variable, ie, pneumatization, is also genetically determined. In fact, it may be deduced that both phenomena are determined by a polygenic system.[16]

Our present study supports the prior view of Albrecht,[17] Schwartz,[18] and Cheatle,[19] who considered hereditary factors to play at least a part in the determination of the various types and sizes of pneumatization. This does not exclude the possibility that other factors, such as inflammation, contribute to and influence the final size of mastoid pneumatization, leaving open the possibility of a multifactorial process.

The origin of the difference in size and type of pneumatization has become highly controversial over the years. The congenital inheritance theory of pneumatization was strongly supported by Diamant[3] and Goto and Nagata,[20] who like their predecessors Mouret,[21] Heine,[22] and Cheatle[19] held the view that compact mastoid bones develop secondarily to a hereditary individual disposition.

The opposing theory that supports an acquired, inflammatory origin as determining the final size of the mastoid system was advocated by Wittmack[1,23] and Tumarkin[24] and lately by Aoki et al[25] and Tos et al.[9] A careful examination of Wittmack's statements reveals, however, that even he actually believed in a dual influence on the final product of pneumatization. Wittmack thought that it is the type of mucous membrane that is decisive for the final size of pneumatization. The type of the mucosa, according to him, was a derivative of whether or not a particular ear was inflamed in infancy. At the same time, however, he believed that hereditary factors are at the root of the mucosal destiny.[23]

The finding that a hypocellular pneumatic system of the mastoid is typical of ears with chronic otitis media was emphasized by both Wittmack[1,23] and Diamant[3] and is generally accepted today. Some authors have claimed that the size of the air cell system also is reduced in children with secretory otitis media.[7,26,27] However, Sadé and Hadas[6] have shown that pneumatization in secretory otitis media is not uniform and may extend from a minimal to a maximal degree. Their study also showed that pneumatization in secretory otitis media is linked to its prognosis: the smaller the mastoid, the worse the prognosis. A similar observation was later reported by Tos et al.[28] However, as in most other studies that show a link between middle ear inflammation or its severity and pneumatization, no conclusions can be drawn as to whether the disease (ie, the duration and severity of the inflammatory process) caused a reduced pneumatization or vice versa.

In spite of a voluminous literature on this subject, concrete evidence of the influence of inflammation on pneumatization is still lacking. Which came first is still an open question. The recent highly interesting experimental animal studies produced by Aoki et al[25] have been performed under drastic conditions that are remote from what happens in human clinical otitis media. Therefore, no conclusions can be drawn from these animal experiments.

From a practical point of view this study indicates that good pneumatization supports the clinical diagnosis of otosclerosis, just as normal otoscopic results and the absence of a history of middle ear inflammatory diseases do. When in doubt, a mastoid radiograph may decide the matter: mastoid pneumatization of less than 7 cm^2 ordinarily excludes otosclerosis. On the other hand, pneumatization of 25 cm^2 or more is highly suggestive of otosclerosis.

From this study it also seems that otosclerosis and any of the chronic otitis media syndromes are almost mutually exclusive. A fixed footplate found in a chronic otitis media is therefore most probably not of otosclerotic origin. Furthermore, the fact pointed out in this study that patients with otosclerosis have extensive pneumatization leads to one of two possibilities:

1. Patients with otosclerosis do not suffer in infancy from acute and secretory otitis media as the rest of the pediatric population does, and therefore develop a large pneumatization. If this is true, then there seem to be some factors that prevent them from contracting otitis media.
2. If patients with otosclerosis suffered from infantile otitis media as does the rest of the pediatric population, then the otitis media in their

cases could have no, or only little, influence on the extent of their pneumatization. This is in contrast to the postulate defended by proponents of environmental theories.

ACKNOWLEDGMENTS — The authors thank Professor A. Adam and Dr M. Bat Miriam for helpful advice on the genetic aspects of this study, and I. Gelenter for her assistance in statistical analysis.

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GROWTH AND CHEMOSENSITIVITY OF HUMAN HEAD AND NECK CANCERS IMPLANTED UNDER THE KIDNEY CAPSULE OF CYCLOSPORINE-IMMUNOSUPPRESSED MICE

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Thirty-seven of 54 human squamous cell head and neck carcinomas were grown successfully as first transplant generation xenografts under the kidney capsule of conventional mice immunosuppressed by daily treatment with 60 mg/kg of cyclosporine. Eighteen different tumors were evaluated for chemosensitivity to cis-diamminedichloroplatinum (cisplatin), 5-fluorouracil, and methotrexate. Thirty-nine percent of the tumors responded to cisplatin, 19% to 5-fluorouracil, and 33% to methotrexate. This assay response is consistent with the clinical response of human squamous head and neck carcinoma to these drugs used as single agents. It is hoped that this model will become useful for new drug testing and, in certain cases, for selection of chemotherapy for patients with refractory tumors.

KEY WORDS — carcinoma xenografts, cyclosporine, drug screening, heterologous transplantation.

INTRODUCTION

We have previously shown that freshly resected human carcinomas can be grown as first transplant generation xenografts under the kidney capsule of immunosuppressed mice.[1] Both congenitally immunosuppressed athymic nude mice and conventional mice immunosuppressed by daily treatment with cyclosporine A (CsA) supported the long-term growth of these human tumor xenografts.[1,2] Tumor growth rates and chemosensitivity profiles in nude mice were similar to those in CsA-treated mice,[2] making it reasonable to substitute CsA-treated mice for nude mice in this model. This substitution was helpful because CsA-treated mice are cheaper and hardier than nude mice. Using this modification of the subrenal capsule assay, tumor growth curves could be established in each mouse by surgical re-exteriorization of the tumor-bearing kidney and measurement of tumor size at weekly intervals. The mice tolerated the daily CsA treatment and additional laparotomies quite well, losing less than 10% of their body weight over a 3- to 4-week interval.[2] Based on this information, it appeared that this model would be useful for testing the chemosensitivity of freshly resected human carcinomas. It was important to select a carcinoma that was moderately responsive to single-agent chemotherapy in order to avoid a study that obtained a positive correlation of tumor responsiveness in the assay to that in the patient through negative results; that is, no tumors chemoresponsive in the assay, and no tumors chemoresponsive in the patient. Squamous cell carcinomas of the head and neck are relatively prevalent tumors[3] that are known to be moderately responsive to single-agent chemotherapy[4-7] and are relatively easy to sample. Thus we elected to study the growth and chemosensitivity of these tumors following implantation under the kidney capsule of mice immunosuppressed by daily treatment with CsA.

MATERIALS AND METHODS

Mice. Male BDF1 mice were purchased from the Jackson Laboratory (Bar Harbor, Me) when they were 7 to 8 weeks old. Mice were not used in experiments until they were 9 to 10 weeks old. Immunosuppressed mice were housed under positive laminar flow and handled by sterile technique. Autoclaved feed and autoclaved, acidified water were provided to all mice ad libitum.

Cyclosporine A. The CsA (Sandimmune IV) was purchased from Sandoz, Inc, East Hanover, NJ, and was diluted in 0.9% NaCl solution for injection. It was stored in glass containers because of its absorption by plastic. Treatment with CsA was begun immediately before tumor implantation. Each mouse received 60 mg/kg/d subcutaneously throughout the course of the experiment.

Procurement of Human Tumor Specimens. Biopsy specimens were obtained at endoscopy or at the time of definitive surgery. Immediately following resection, tumors were sectioned in the surgical pathology laboratory adjacent to the operating room suite under the guidance of a surgical pathologist in order to select tissue that contained a maximum of viable tumor cells and a minimum of necrotic tissue, stroma, and nonneoplastic cells. In many cases frozen sections were examined microscopically to ensure an adequate yield of tumor cells in the piece selected for the subrenal capsule assay. Once the tumor piece had been selected, it was placed in a

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REPRINTS — James A. Bennett, PhD, Me-514 Albany Medical College, Albany, NY 12208.

TABLE 1. ATTAINMENT OF HUMAN SQUAMOUS HEAD AND NECK CARCINOMA GROWTH UNDER KIDNEY CAPSULE OF CYCLOSPORINE-IMMUNOSUPPRESSED MICE

Selection Criteria	*Tumor Cases Grown/ Tumor Cases Implanted*	%
All cases	37/54	68
No prior irradiation	26/37	70
Prior irradiation	11/17	65
Greater than 50% tumor in sample	13/15	87
Less than 50% tumor in sample	1/6	17

sterile specimen container, immersed in Hanks' balanced salt solution containing penicillin (100 U/mL) and streptomycin (100 μg/mL), and transported to a laboratory within the same building for further dissection and implantation under the kidney capsule of mice. The time between removal of tumor from the patient and completion of implantation into mice was less than 3 hours.

The clinical record of each patient was reviewed to determine the primary site, stage, histologic differentiation, and prior therapy.

Implantation of Tumors. The method of implanting human tumors under the kidney capsule of mice has been described by Bogden et al.[8] Mice were anesthetized with Nembutal. The mouse kidney was exteriorized surgically, an incision was made in the capsule, and a tumor fragment measuring approximately 1.5 mm in diameter and less than 1 mm in thickness was implanted under the capsule with the use of a 16-gauge x 3.81-cm (1½ in) trocar. The size of the implant was determined in situ by measurement of two perpendicular diameters to the nearest tenth of a millimeter by means of a dissecting microscope equipped with an ocular micrometer. Ten ocular micrometer units were calibrated to be equivalent to 1 mm. Tumor sizes are reported as average diameter in millimeters. Following measurement of tumor size, the tumor-bearing kidney was returned to the peritoneal cavity, and the wound was closed with silk and wound clips. Each individual mouse was marked for identification. At various times after implantation, the tumor-bearing kidney was exteriorized and a measurement of xenograft size was taken. Xenograft depth and neovascularization also were noted, but were not used in the calculation of tumor size. At the end of each experiment, a final measurement of xenograft size was taken, and the xenograft-bearing kidney was resected and placed in formalin. Preparation of slides for histologic examination was done by cutting a cross section perpendicular to the long axis of each kidney, approximately 3 mm in thickness, containing the tumor tissue. Each section was embedded in paraffin, serially sectioned by microtome, mounted on glass slides, and stained with hematoxylin and eosin.

Chemotherapy. Chemotherapy was initiated only in mice with growing tumors; growth was verified at laparotomy 14 days after tumor implantation. Mice with growing tumors were distributed into a control group and as many as three chemotherapy treatment groups containing three to five mice each. Chemotherapy was administered from the 16th to the 20th day after tumor implantation. Mice were treated intravenously via tail vein injection with either cis-diamminedichloroplatinum (cisplatin; 1.5 mg/kg/d), 5-fluorouracil (20 mg/kg/d), or methotrexate (4 mg/kg/d). These drugs were chosen because of demonstrated activity as single agents against squamous carcinoma of the head and neck.[4-7] The doses and schedules used in the mice were maximally effective regimens against experimental tumors, according to data from the Drug Screening Program at the National Cancer Institute.[9] These treatment regimens also appeared to be close to the maximally tolerated dose in mice, since mice that received them lost 10% to 15% of their body weight.

RESULTS

Fifty-four separate specimens from patients with squamous carcinoma of the head and neck were implanted under the kidney capsule of conventional mice immunosuppressed by daily treatment with 60 mg/kg of CsA. Overall, an average of 12 mice per patient tumor were implanted with tumor. The number of mice implanted with a patient's tumor was determined largely by the amount of tumor obtained at surgery and by the decision of whether the chemosensitivity of that tumor would be evaluated. In cases in which chemosensitivity was evaluated, an average of 20 mice per patient tumor were implanted with tumor. As shown in Table 1, in 37 of 54 different patient tumors, neovascularization and growth of the tumor xenograft was noted 14 to 21 days after implantation. Histologic confirmation of squamous carcinoma in the subrenal capsular space was obtained in each of these cases. For each of the 37 patient tumors grown under the kidney capsule of mice, an average of 73% of the mice grew that tumor. For each of the 17 patient tumors that did not grow under the mouse kidney capsule, none of the mice grew that tumor.

Mice with growing tumors underwent weekly laparotomies to monitor tumor growth. Mice tolerated three to four laparotomies without any gross evidence of deterioration such as weight loss, ruffled fur, decreased body temperature, or decreased motor activity. Repeated measurements of tumor size as a function of time in the same mouse permitted estimation of a tumor growth curve. Typical tumor growth curves are shown in Fig 1, and the slopes of the tumor growth curves for all 37 different patient tumor xenografts are shown in Table 2. Most of the slopes were between 10 and 100, with a clustering around 50. There were three

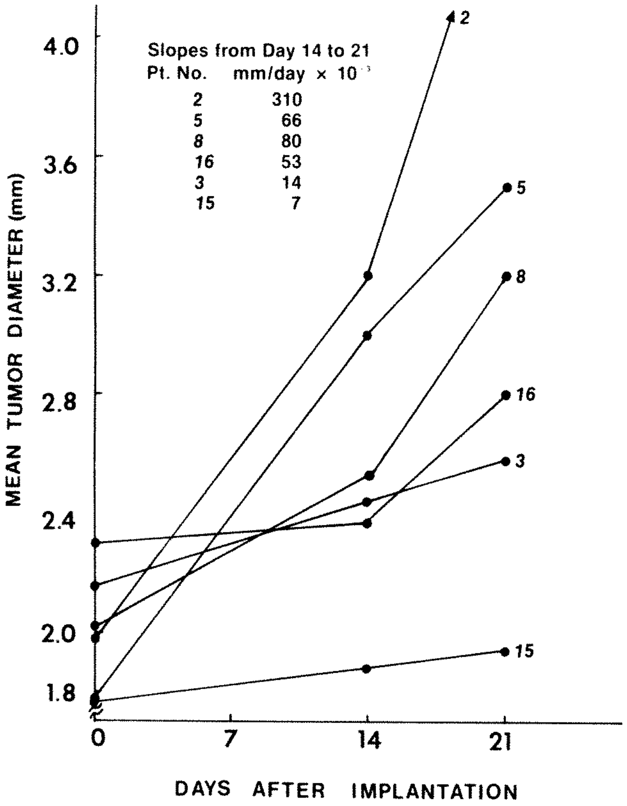

Fig 1. Representative growth of human squamous carcinomas of head and neck following implantation under kidney capsule of mice immunosuppressed by cyclosporine A. Average tumor diameter was measured in each mouse on day of tumor implantation and again 14 and 21 days after tumor implantation. Each point is mean tumor diameter in three to five replicate mice.

cases of unusually rapid growth and two cases of unusually slow growth. All three cases of unusually rapid tumor growth in the assay were associated with rapid tumor growth and early death (less than 6 months from the time of surgery) of the patients.

Both patient and cancer specimen variables were reviewed for possible correlation with successful growth in the assay. Tumor stage, degree of tumor differentiation, primary anatomic site, biopsy site, and previous treatment were evaluated as predictors of tumor take. Unexpectedly, none of these correlated with tumor take. Even in patients who had received prior irradiation, tumor take was similar to that found in patients who had not received prior irradiation (Table 1). This was unexpected, since irradiation is known to increase the amount of necrotic and fibrous tissue in the tumor. Such a change should have decreased the probability of obtaining highly viable homogeneous tumor tissue for the assay, which in turn should have led to decreased tumor takes in the assay. The similarity in tumor takes between previously irradiated and nonirradiated patient tumors suggests that the procedure used by the surgical pathologist to select tumor tissue for the assay avoided those areas of the tumor heavily infiltrated with nonviable and fibrous tissue. In 21 cases, after implantation of tumor pieces in the assay, sufficient tumor sample remained for fixation and subsequent histologic evaluation. As shown in Table 1, tissue samples containing a high percentage of tumor cells (greater than 50%) had substantially higher takes than samples containing a low percentage of tumor cells (less than 50%). Thus, of all the variables that we examined, tumor cellularity of the specimen was the only one that correlated with take in the assay.

TABLE 2. GROWTH RATES OF HUMAN HEAD AND NECK SQUAMOUS CARCINOMAS UNDER KIDNEY CAPSULE OF CYCLOSPORINE-IMMUNOSUPPRESSED MICE

Patient No.	*Slope of Tumor Growth Curve (mm/d x 10^{-3})*
1*	19 ± 7
2	310 ± 40
3	14 ± 7
4*	88 ± 41
5	66 ± 4
6	23 ± 2
7	116 ± 20
8*	80 ± 9
9	13 ± 4
10	63 ± 22
11	12 ± 10
12	16 ± 5
13*	38 ± 19
14	50 ± 9
15	7 ± 10
16	53 ± 8
17*	94 ± 20
18	44 ± 19
19*	180 ± 75
20	80 ± 10
21*	61 ± 20
22*	47 ± 26
23	13 ± 6
24	83 ± 5
25†	25
26†	36
27	33 ± 11
28	9 ± 5
29*	55 ± 9
30	48 ± 8
31*	74 ± 15
32	11 ± 5
33*	52 ± 26
34	53 ± 12
35†	39
36	34 ± 18
37	35 ± 6

Pieces of freshly resected human squamous carcinoma of head and neck were implanted under kidney capsule of immunosuppressed mice. Tumor size in each mouse was measured on days 14 and 21 and, in some cases, days 28 and 35. Slopes were calculated by subtracting average tumor diameter on day 14 from average tumor diameter on day 21 and dividing by time interval of 7 days. Values are mean tumor diameters ± standard error obtained in three to five replicate mice per tumor.

*Tumors did not increase in size until third or fourth week after implantation. In these cases, first measured increase in tumor size was subtracted from size measured 1 week later and divided by 7-day time interval.

†Tumor grew in less than three replicate mice.

As already demonstrated in Fig 1 and Table 2,

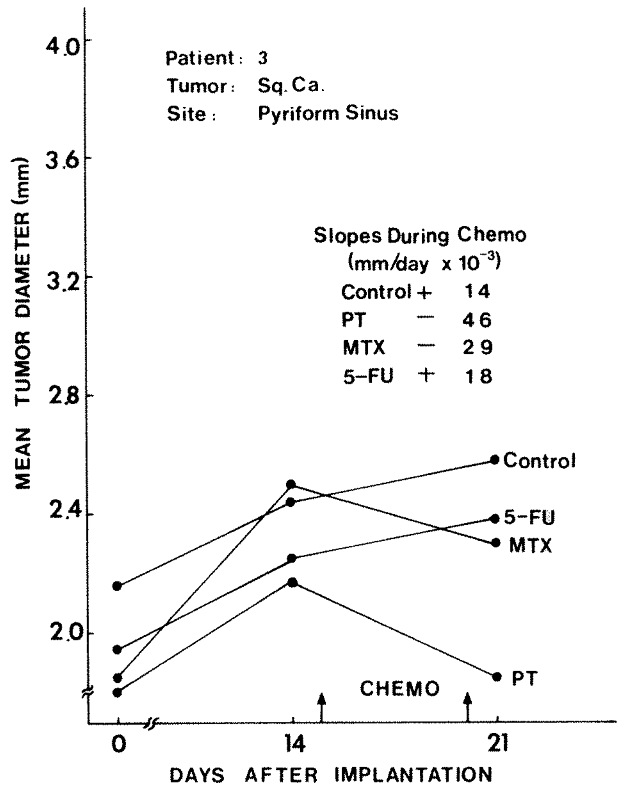

Fig 2. Representative chemosensitivity profile of human squamous carcinoma (Sq. Ca.) of pyriform sinus growing under kidney capsule of mice immunosuppressed by cyclosporine A. Footnote to Table 3 explains drug treatment and calculation of slopes. Chemo — chemotherapy, PT — cisplatin, MTX — methotrexate, 5-FU — 5-fluorouracil.

TABLE 3. EFFECT OF CHEMOTHERAPY ON HUMAN HEAD AND NECK CANCERS GROWING UNDER KIDNEY CAPSULE OF CYCLOSPORINE-IMMUNOSUPPRESSED MICE

	Growth Rate (mm/d x 10^{-3})			
Patient No.	*Control*	*Cisplatin*	*5-Fluorouracil*	*Methotrexate*
2	310±38	180±22	170±41	340±70
3	14± 7	− 46±15	18±12	− 29±18
4	88±41	12± 8	20±12	12± 7
5	66± 4	43±10	43± 7	79±17
12	16± 5	18±10	26± 4	
15	7±10	− 19±22	− 34±13	
16	53± 8	− 9±10	69±35	
17	94±20	6± 3		97±19
18	44±19	19±42		− 43±10
19	180±75	164±20	190±13	
20	80± 9	28± 4	36± 6	28±24
21	210±24	110±20	164±37	
22	47±26	45± 8	− 8± 9	
29	55± 9	− 15±10	31± 6	
31	19± 3	− 14± 7	17±10	− 26±10
34	53±12	− 56± 6	− 10±10	
36	34±18	24±10	1± 5	16± 6
37	35± 6	− 10±10	6± 7	

Mice growing a patient's tumor were divided into groups containing three to five mice per group. Each group received one of above chemotherapeutic agents or saline intravenously at doses indicated in Methods section every day for 5 days. In most cases this entailed administration of chemotherapy from 16th to 20th day after tumor implantation. Slopes were calculated by subtracting mean tumor diameter measured 2 days before initiating chemotherapy from mean tumor diameter measured 1 day after cessation of chemotherapy and dividing by 7-day time interval. Values are mean tumor diameter ± standard error obtained in three to five replicate mice per tumor.

serial measurements of tumor xenograft size at weekly laparotomies provided data that made it possible to draw tumor growth curves and calculate tumor growth rates. This permitted evaluation of the sensitivity of tumors to chemotherapeutic agents. This was done by administering cancer chemotherapeutic agents to groups of mice with growing tumors and measuring drug response by comparing tumor growth in drug-treated mice to that in non-drug-treated mice. We evaluated the sensitivity of 18 of these tumors to cisplatin, 5-fluorouracil, and methotrexate. The results of a typical chemosensitivity profile are shown in Fig 2, and the slopes of the tumor growth curves for all 18 patients are shown in Table 3. A response in the assay was defined arbitrarily as tumor regression in the drug-treated group with continued tumor growth in the non-drug-treated group. This was a more rigorous definition of response than a statistically significant difference in growth between the control and drug-treated groups, since in some cases drug treatment resulted in a statistically significant inhibition in tumor growth but not tumor regression. In nine of 18 cases at least one drug shrunk the tumor, and in four of 18 cases more than one drug shrunk the tumor. Seven of 18 tumors (39%) responded to cisplatin, three of 16 tumors (19%) responded to 5-fluorouracil, and three of nine tumors (33%) responded to methotrexate. Failure to respond to cisplatin did not preclude response to other agents. These data indicate that cisplatin and methotrexate were more effective than 5-fluorouracil as single agents in shrinking squamous carcinomas of the head and neck. As shown in Table 4,[4-7] this is consistent with the known clinical responsiveness of head and neck cancers to these drugs used as single agents.

DISCUSSION

This study demonstrated that freshly resected human squamous carcinomas of the head and neck can be grown readily as first transplant generation xenografts under the kidney capsule of mice immunosuppressed by daily treatment with 60 mg/kg of CsA. A primary take rate of 68% was achieved, and for each patient tumor implanted under the kidney capsule of a group of mice, an average of 73% of the mice grew that tumor.

Human squamous carcinomas of the head and neck have been grown in a variety of ways. Clonogenic assays in vitro and xenografting in vivo have been the most common. Less than 50% of head and neck cancer specimens grew in clonogenic assays, and adequate numbers of cells were available for chemosensitivity testing in less than 20% of these

TABLE 4. COMPARISON OF HISTORICAL RESPONSIVENESS TO ASSAY RESPONSIVENESS OF HUMAN SQUAMOUS HEAD AND NECK CANCER TREATED WITH SINGLE-AGENT CHEMOTHERAPY

	Historical			
Drug	*No Previous Treatment*	*Irradiation Recurrence*	*Chemo-therapy Recurrence*	*Assay*
Cisplatin	40%	25%	5%-15%	41%
5-Fluorouracil	25%	15%	5%-15%	20%
Methotrexate	35%-50%	25%-35%	15%-20%	38%

Historical data from references 4 through 7.

cases.[10,11] Similar outcomes have been obtained with xenografting. Elprana et al[12] were able to grow ten of 13 different head and neck cancers in the subcutaneous space of nude mice with an average take rate per mouse of 31%. Wennerberg et al[13] have grown 24 of 85 different head and neck cancers in the subcutaneous space of nude mice. The basis for the discrepancy in take rate between these two studies is not clear, but may be related in part to the fact that the former study used two to three times more mice as recipients of first transplant generation xenografts than the latter study. In comparison, a higher overall take rate was found in our study. This may be due to the fact that the subrenal capsular space is supplied by a rich vascular bed providing an immediate wealth of nutrients to tumors placed in that space. It is also possible that CsA-treated conventional mice are immunosuppressed more effectively than athymic nude mice for xenograft growth. It has been reported that nude mice have some residual competence to recognize foreign grafts[14,15] and that sublethally irradiated nude mice are more efficient in growing foreign grafts than nonirradiated nude mice.[16]

Other investigators[17,18] have placed squamous carcinomas of the head and neck in the subrenal capsular space for the purpose of chemosensitivity testing. However, all of these studies used immunocompetent rather than immunosuppressed mice and a 6-day time frame in which to carry out the study. Although this provides a more rapid, less expensive, and less cumbersome model for chemosensitivity testing than the model described in the present study, there are drawbacks to using immunocompetent mice and such a short time frame. First, most human solid carcinomas grow too slowly to manifest significant growth during the first 6 days after implantation. Second, in immunocompetent mice host immune cells begin to infiltrate the xenograft 3 to 4 days after implantation, leading to graft rejection 6 to 7 days after implantation.[1,18,19] Third, in such a model the target of a chemotherapeutic agent could be the human tumor component, human noncancerous tissue inadvertently implanted with human tumor, the murine host defense component, or a combination of these components, thereby complicating interpretation of drug-induced tumor toxicity in the assay.

In the present study a minimum of 2 weeks was given for the tumor to establish a blood supply and grow. Chemotherapy was not initiated until tumor growth was verified at laparotomy. A group of tumor-bearing mice not receiving chemotherapy was maintained as a control against which drug-treated groups could be compared. This is the first demonstration of serially monitoring tumor growth under the mouse kidney capsule in this way. Under these conditions the chemosensitivity profiles that were obtained compared reasonably well to the historical responsiveness of this tumor to single-agent chemotherapy.

There are problems inherent in the design of a prospective clinical trial that would test the predictive accuracy of this assay as well as other chemosensitivity assays. It would be difficult to mimic drug schedules and drug combinations used clinically. Differences in tumor burden, tumor location, and vascular access between tumor in the patient and tumor growing as a xenograft may cause discrepancies. Also, there would be differences in definition of drug response. However, in spite of these drawbacks, the model described in this study provides the advantages of high tumor take rate, generation of tumor growth curves, and assurance that chemotherapy is being delivered into an in vivo system against a verified growing tumor. With these advantages it is hoped that this model will become useful for new drug testing, for evaluating the biochemical pharmacology of therapeutically useful drugs and drug combinations, for evaluating the mechanisms for development of drug resistance, and perhaps in certain cases for selection of chemotherapy for patients with refractory tumors.

ACKNOWLEDGMENTS — The authors thank Vernon A. Pilon, MD, for histopathologic evaluation of tumors and Janet H. Peters for help in implanting tumors under the kidney capsule of mice.

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CONFERENCE ON THE EUSTACHIAN TUBE AND MIDDLE EAR DISEASES

The Conference on the Eustachian Tube and Middle Ear Diseases will be held October 26-29, 1989, in Geneva. For further information, contact Prof Jacob Sade, Chairman, c/o The Secretariat, Conference on the Eustachian Tube and Middle Ear Diseases, Intercontinental Hotel Geneva, 7-9 CH du Petit Saconnex, CH-1211 Geneva 19, Switzerland.

Ann Otol Rhinol Laryngol 98:1989

COCHLEAR HAIR CELL STEREOCILIA LOSS IN LP/J MICE WITH BONE DYSPLASIA OF THE MIDDLE EAR

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LP/J inbred mice spontaneously develop bony lesions of the middle ear and otic capsule that are similar to those of human otosclerosis and tympanosclerosis. These mice also have progressive loss of hearing due to cochlear hair cell loss. The purpose of this study was to describe quantitatively the deterioration and loss of cochlear hair cells to serve as a basis for future experiments attempting to alter the course of this disorder. Cochleas from 37 LP/J inbred mice were examined by scanning electron microscopy. The stereocilia loss in the cochlea was evident as early as 15 weeks of age and progressed from the basal turn to the apex. Outer hair cells were affected more than inner hair cells. As outer hair cells deteriorated we observed fusion, bending, and breakage of stereocilia. There were no apparent differences in the mode of deterioration among the three rows of outer hair cells. Stereocilia fusion of inner hair cells occurred at an older age, and giant, elongated stereocilia were found in some of the animals.

KEY WORDS — bone dysplasia, hair cell loss, LP/J mice, otosclerosis, stereocilia, tympanosclerosis.

Inbred LP/J mice develop a unique disease of the middle ear that manifests as bony, exophytic lesions within the middle ear space.[1] These lesions have been compared to those in human otosclerosis and tympanosclerosis, and the animals progressively lose auditory function with age.[2] In addition to the middle ear lesions that may cause conductive auditory deficits, these animals also have cochlear hair cell loss.[1,2] Both the middle ear disease and the hair cell loss appear to be genetically determined and to follow a recessive mode of inheritance.[3]

The relationship between human otosclerosis and cochlear hair cell loss is not clear. Most studies indicate that patients with otosclerosis experience greater deterioration of cochlear function than do age-matched controls. The relationship between lesions of the bony otic capsule and cochlear deterioration is unknown. Since LP/J inbred mice have both dysplastic otic capsular lesions and progressive cochlear hair cell loss,[1,2] a careful study of these animals may provide some insight into the cause of cochlear damage in human otosclerosis. The purpose of this study was to describe quantitatively the deterioration and loss of cochlear hair cell stereocilia in LP/J inbred mice at various ages to serve as a basis for future experiments attempting to alter the course of this disorder.

METHODS AND MATERIALS

Thirty-five LP/J inbred mice reared in our colony were used for this study. The animals were first and second generation progeny of breeding stock obtained from the Jackson Laboratory, Bar Harbor, Me, and were raised under quiet conditions. Each animal was killed painlessly by intraperitoneal injection of pentobarbital sodium and its bulla was dissected. The cochleas were perfused by gently injecting 2% paraformaldehyde, 2% glutaraldehyde in 0.08 *M* cacodylate buffer at 4°C into the oval window until fixative flowed out of a small opening made at the apex of the cochlea. The fixative was infused within 1 minute after death. After fixation for 1 hour the cochleas were washed with buffer and postfixed with 1% OsO4 for 1 hour at 4°C. The cochleas were dissected under water with use of a binocular dissecting microscope. While the cochlea and adjacent bone were stabilized with toothed forceps, small fractures were made into the bony wall of the cochlea with a sharp pick. The dissection was facilitated by immersing the cochlea in clear buffer solution; a gentle stream of liquid was used to flush away bone chips. Larger bone chips were removed with fine-point forceps. After removal of the lateral bony wall, the spiral ligament and the stria vascularis were removed with sharpened forceps with the organ of Corti left in place.

After dissection, specimens were washed in distilled water and placed in a saturated solution of thiocarbohydrazide (TCH) for 30 minutes. After washing again, the specimens were placed in 1% OsO4 for 2 hours; the TCH and OSO4 steps then were repeated twice: the OTOTO method.[4] The specimens were washed in distilled water, dehydrated in graded solutions of acetone, and critical point dried. The mounted specimens were screened on an ISI-DS130 scanning electron microscope at 700x, and each available region of the organ of Corti was photographed.

Hair cell counts were derived by counting the presence or absence of each inner hair cell (IHC)

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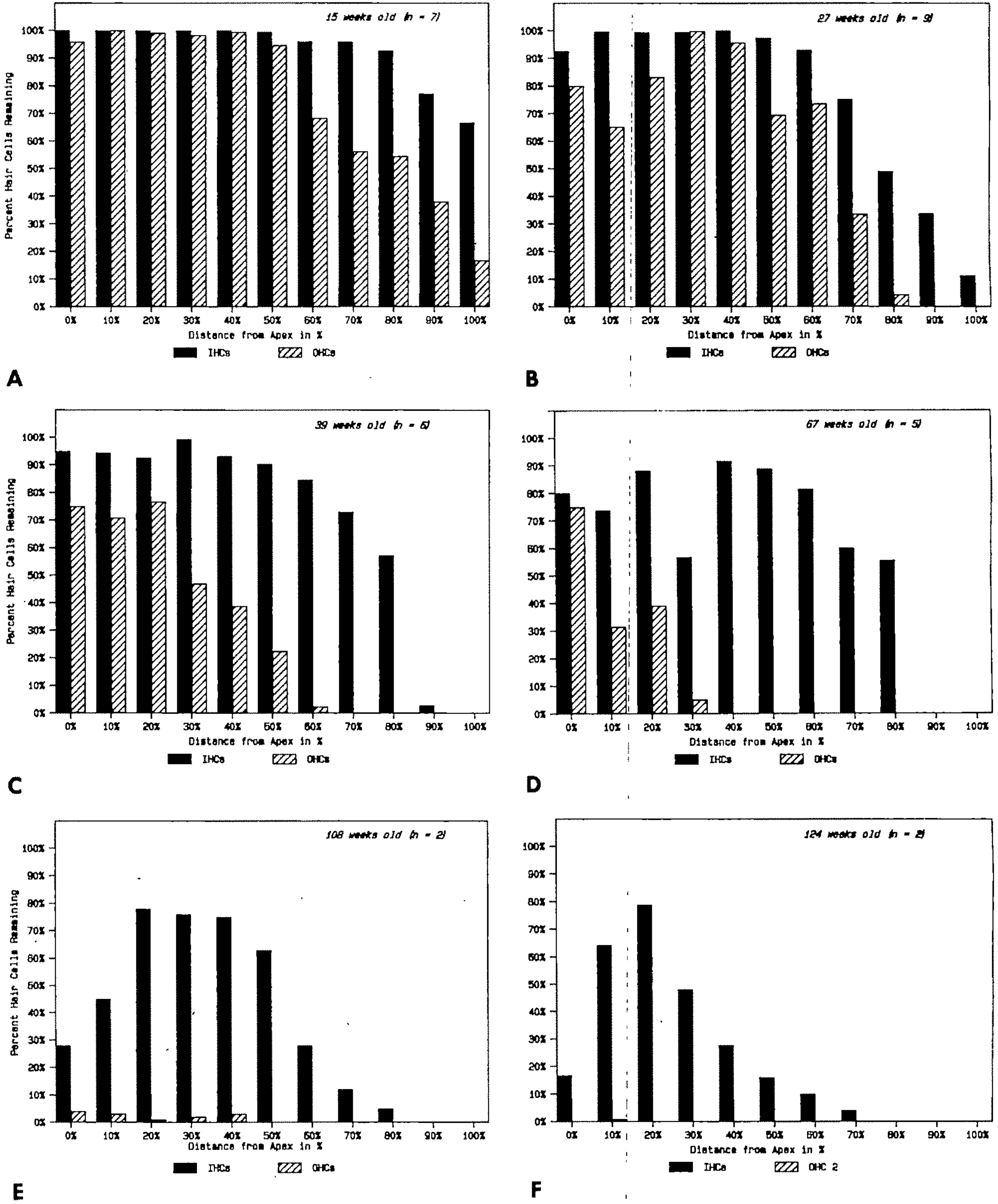

Fig 1. Cytocochleograms obtained from cochleas of animals at A) 15 weeks, B) 27 weeks, C) 39 weeks, D) 67 weeks, E) 108 weeks, and F) 124 weeks of age.

and outer hair cell (OHC). A cell was counted as "missing" if there were no identifiable stereocilia on the surface. The percentage of hair cells remaining in each of 11 areas of equal size along the basilar membrane was calculated and a cytocochleogram was made. In each group, average cytocochleograms were obtained from several cochleas. Sections of the organ of Corti sometimes were missing from a given specimen and were discounted for the purposes of deriving averaged cytocochleograms.

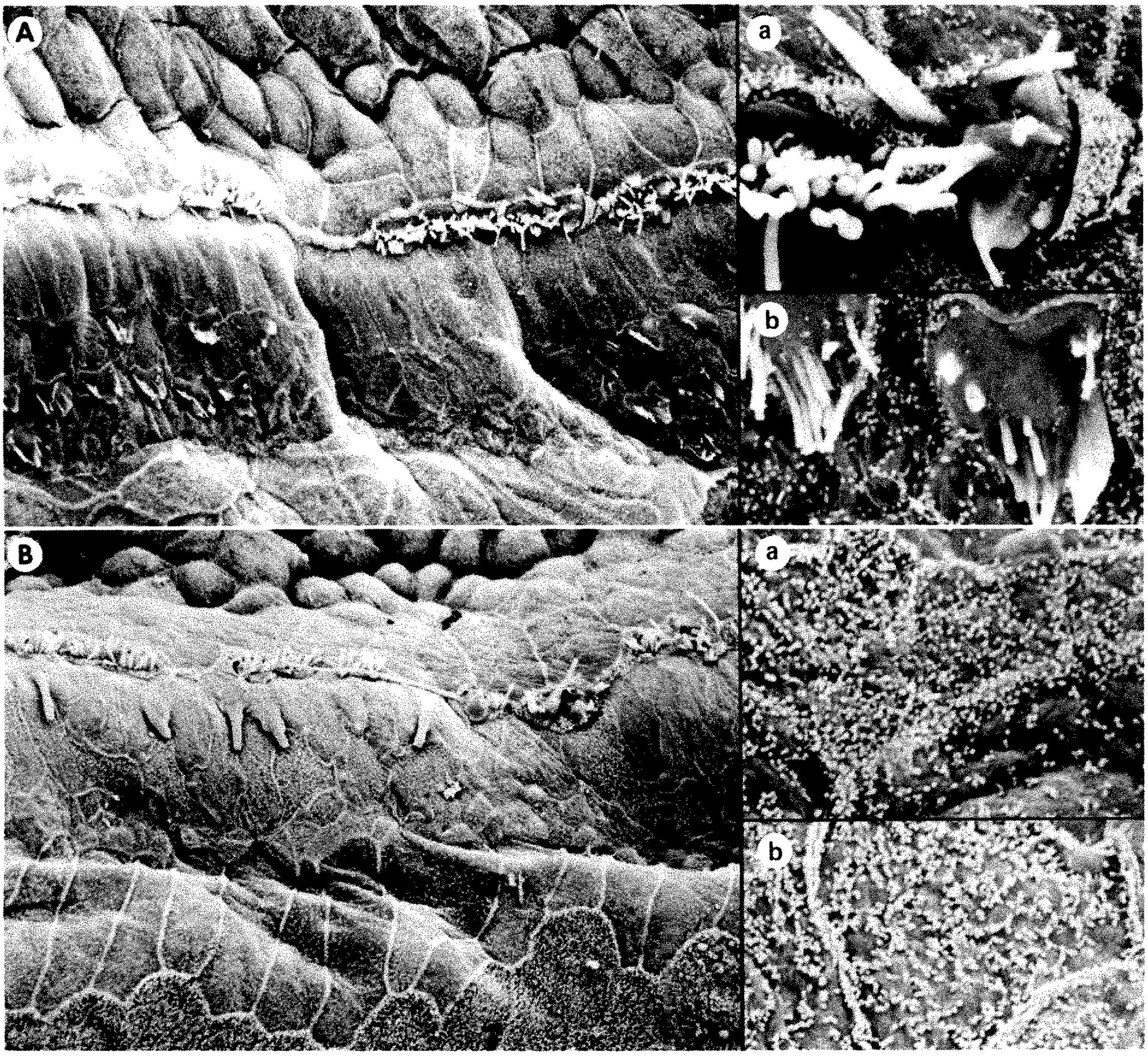

Fig 2. Scanning electron micrographs of mid-cochlear hair cells. A) Moderate loss in 39-week-old animal (original x1,500). a — inner hair cell area, b — outer hair cell area (originals x5,000). B) Severe loss in 127-week-old animal (original x1,500). Giant stereocilia and fused stereocilia are seen. a — inner hair cell area, b — outer hair cell area (originals x5,000).

RESULTS

No cochlear hair cell loss was seen in the 1-week-old animals or in the 5-week-old animals. In the 15-week-old animals, hair cell loss was found in the basal turn (Fig 1A). The loss of OHCs was far greater than the loss of IHCs. For the most part, missing hair cells were replaced by phalangeal scars (Fig 2A), but distortions of stereocilia of the OHCs were common. Those distortions were more prevalent in the older animals (Fig 2). A variety of specific abnormalities of OHC stereocilia were seen with increasing age. Fusion of stereocilia was present in some areas as early as 5 weeks. Later changes in OHC stereocilia included bending and loss of stereocilia (Fig 2A). Inner hair cell stereocilia tended to deteriorate later than did OHC stereocilia. Damage in IHC stereocilia included stereocilia fusion with confluence of the limiting membrane (Fig 3) and giant stereocilia (Fig 2B). Loss of IHC stereocilia was a late event.

Cochlear hair cell loss increased with age. At 27 weeks of age the basal-most portion of the cochlea was devoid of OHCs but some IHCs remained (Fig 1B). Loss of IHCs and OHCs progressed in the remainder of the age groups studied (39, 67, 108, and 124 weeks) (Fig 1C-F). The pattern of hair cell loss was consistent: OHC loss was always more severe than IHC loss and the basal end of the cochlea was more affected than the middle and apical areas. At 108 and 124 weeks of age (Fig 2E,F) apical loss of IHCs was more severe than loss in the middle turn; OHCs were almost completely lost at these ages.

All three rows of OHCs appeared to deteriorate at the same rate with age (Fig 4).

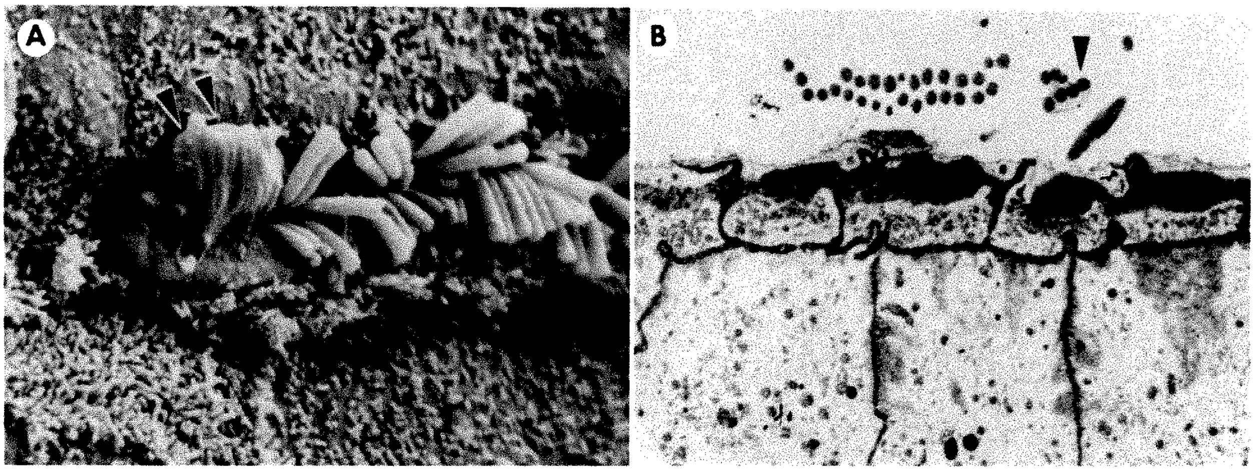

Fig 3. Fusion of inner hair cell stereocilia (original x5,200). A) Scanning electron micrograph demonstrating fusion of stereocilia (arrowheads) and bending of adjacent stereocilia. B) Transmission electron micrograph of similar area demonstrating confluence of limiting membrane around these fused cilia (arrowhead).

DISCUSSION

LP/J inbred mice exhibit progressive loss of auditory function with age. The anatomic correlates of this loss are middle ear bony lesions and cochlear hair cell loss.[1-3,5] Both the middle ear disease and the hair cell loss appear to be genetically determined and follow a recessive mode of inheritance.[3] Hair cell loss has been estimated previously by light microscopic surface preparation techniques[1]; in that study significant loss of cochlear hair cells did not occur until after 15 weeks, an age at which bony lesions may be present.[5] In the present study we found a more extensive loss of IHC and OHC stereocilia than in the previous study. This change is due to differences in the structures being counted in the two studies. In the previous study, remaining cell bodies were counted because stereocilia in mice are extremely difficult to see by light microscopy. In this study we used scanning electron microscopy and counted hair cells as present if they had stereocilia and absent if the stereocilia were missing.

The progression of hair cell loss in the cochlea of these animals precedes the bony lesions of the middle ear and otic capsule.[5] Therefore, it is unlikely that the dysplastic bony lesions in LP/J mice directly cause the observed cochlear hair cell loss. Furthermore, the bony lesions frequently are found on the ossicles and rarely are found in the otic capsule.[5]

The pattern of hair cell loss in LP/J mice is similar to that of aging chinchillas[6] in that the OHCs deteriorate before the IHCs do and the loss occurs in the base before it does in the apex. The chinchilla, however, has far less hair cell loss with age than does the LP/J mouse. In contrast, the rat cochlea[7] deteriorates with age more in the apex than in the base, and the loss of IHCs and OHCs is about equal. Age-related losses in the guinea pig[8] also manifest

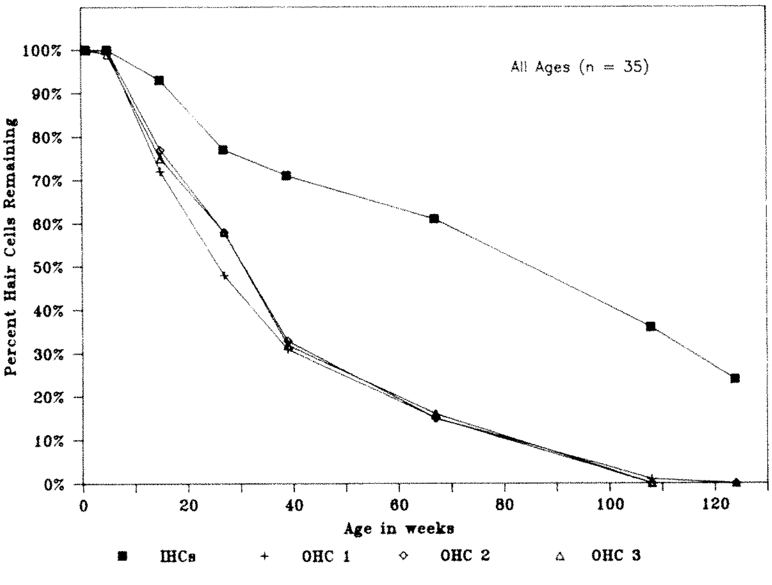

Fig 4. Hair cell loss as function of age.

as loss of apical OHCs, although IHCs are relatively unaffected. The pattern of IHC and OHC loss in aging LP/J mice is similar to that in other inbred mice, although differing in degree.[9]

On the basis of the facts that hair cell loss in LP/J mice does not follow the development of bony lesions temporally and that the pattern of hair cell loss parallels that in other aging rodents, it is unlikely that the bone disease causes the cochlear damage in these animals; however, both the hair cell loss and the dysplastic lesions may have a similar cause.

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AMERICAN AUDITORY SOCIETY

The 16th annual meeting of the American Auditory Society will be held in New Orleans on Sunday, September 24, 1989. The meeting is scheduled in conjunction with the annual meeting of the American Academy of Otolaryngology - Head & Neck Surgery. For further information, contact J. W. Hall III, PhD, Department of Otolaryngology, School of Medicine, Vanderbilt University, Nashville, TN 37232.

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CYLINDRICAL COCHLEAR ELECTRODE ARRAY FOR USE IN HUMANS

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This report describes the fabrication of a flexible multichannel electrode array suitable for use in humans. The conductors, pads, and stimulating tips are made of platinum on a polyimide substrate. Photolithographic techniques are employed in the fabrication of the electrode on a planar surface in the form of a film. The film is rolled subsequently into a cylinder of diameter 0.50 mm and the cylinder is filled with medical grade silicone rubber. The stimulation pads then form rings around the cylinder. In vitro and in vivo tests are ongoing with good results so far.

KEY WORDS — flexible multichannel cochlear electrode.

INTRODUCTION

The development of an implantable prosthetic device for humans with failed inner ear function has become feasible with the increased sophistication of microelectronic technology. It is now possible to have on a single silicon chip enough information-processing power to mimic, in a somewhat crude fashion, the processes that take place in the healthy ear.

The system consists of a microphone connected to a speech signal processor that extracts some of the most important features of the signal and transforms them into a form suitable for input to an electronic neural stimulator. The neural stimulator is implanted behind the ear and anchored to the mastoid bone.[1,2] It is connected to an electrode array, the tip of which is placed in the cochlea. Electric signals from the stimulator directly stimulate the nerve endings in the cochlea and cause the signals to travel down the acoustic nerve fibers to the brain, where they produce a sensation of hearing.

This paper is concerned with the design and manufacture of an electrode array that not only must survive the process of implantation but must function properly for 20 to 25 years. The manufacturing techniques must be, as far as possible, compatible with mass production techniques, which will ensure both reliability and low cost.

ELECTRODE PROPERTIES

The physical dimensions of the electrodes are dictated by the size of the scala tympani. Measurements taken from cadaver cochleas[3,4] show that a diameter as small as about 0.50 mm is required if trauma to the soft tissue in the cochlea is to be avoided during the insertion. At a very early stage a decision was made to develop a multichannel cochlear implant to exploit the tonotopic organization of the cochlea. The question was then how many channels could be accommodated within the scala tympani, given that each electrode in the array must have a minimum size. An array with nine single electrodes was chosen as a practical number to aim for. The development of the electrode fabrication process could begin with the knowledge that the number of channels and the actual configuration of the electrode could be changed at a later date if deemed necessary.

To minimize current spread and thresholds, it is necessary to place the electrode tips as close as possible to the section of the basilar membrane corresponding to the frequency range 300 Hz to 3 kHz. The surface area used in the stimulation must be small enough to fit into the space available and excite only a discrete population of targeted neurons but at the same time large enough to keep the current density at a safe level.[5] The impedance of the electrode, which is a function of the electrode area, must be kept at a value compatible with the range of voltages that the neural stimulator can be expected to supply.

It has been determined that the electrode must penetrate a distance of about 25 mm from the round window.[6] In addition, it was advantageous to make a direct connection between the electrode and the stimulator (to be mounted on the mastoid) to avoid problems that would arise from the use of a ribbon connector between them. The overall length of the electrode array was chosen to be 70 mm. Effectively, the proposed electrode is a combined electrode and ribbon connector.

The success of the implant operation depends to a large degree on the electrode having the correct combination of stiffness and flexibility: stiff enough for the surgeon to be able to push it into the scala tympani and yet flexible enough for it to follow the spiral shape of the cochlea without doing damage to the soft tissue.

It is of the utmost importance that the electrode be inert to the environment of the scala tympani;

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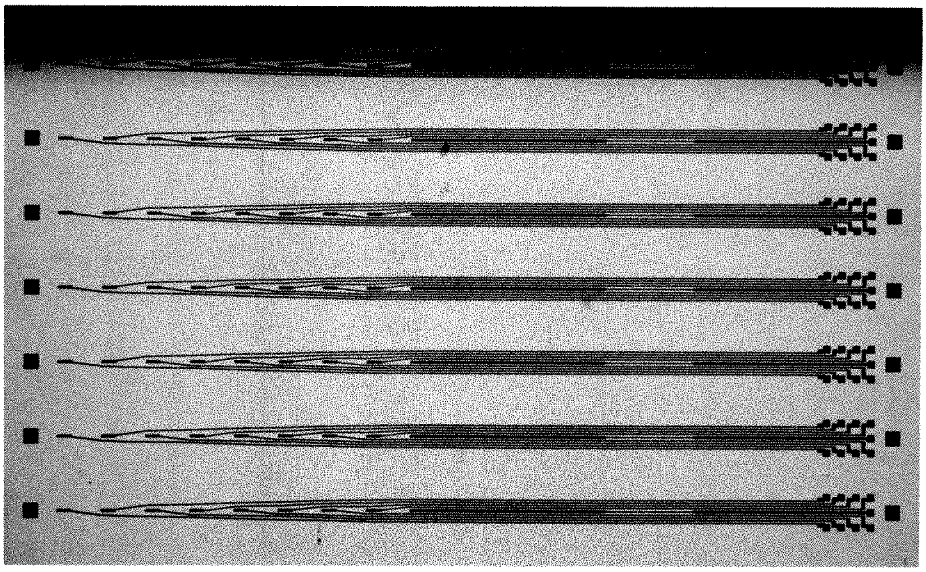

Fig 1. Planar electrode. Mask shows stimulation tips (0.965 x 0.05 mm) (on far left of each diagram, small dark rectangles, and proceeding to right, nine in all), connection pads (nine black squares on far right of each diagram), and feedlines connecting them (0.05 mm wide).

that is, the materials used for making the electrode must be biocompatible. The metal used as the conductor must be highly resistant to corrosion when subjected to electric stimulation in the particularly harsh chemical environment of the cochlea. The insulating material that holds the conductors together must not absorb the perilymph or leach out any foreign matter.

ART OF ELECTRODE MANUFACTURE

The first recorded use of an electrode was by Volta[7] in the year 1800. He employed it in stimulating his own eardrum. More recent electrodes designed for use in cochlear implants can be classified into two broad groups. The first type is designed to go into the cochlea and therefore must be flexible. The second makes direct contact with the auditory nerve at a point at which the nerves leave the cochlea, and the electrodes in this group can be stiff.[8,9] The electrodes can be classified further according to the method of manufacture—those that are handmade[10-13] and those that are made by mass production.[14-17]

In terms of reliability and cost, electrodes made by photolithography are superior to handmade ones. However, those that have been made so far have shown some severe drawbacks.

1. They are planar and therefore all the stimulating pads have to be on one side of the plane; thus the space available for each pad is limited.
2. It is rather difficult to insert a long, thin, flat structure into the spiral-shaped cochlea.
3. They tend to delaminate since they are made in the form of a sandwich with platinum between two layers of insulating material.
4. They have been found to be toxic to tissue, mainly because traces of the highly corrosive etchants remain on the surface or are absorbed by the insulating material in spite of prolonged rinsing in water.

Because of the above difficulties, all the currently available cochlear prostheses use handmade electrodes.

This paper discusses the development of an electrode that is flexible, nontoxic, reliable, and circular in cross section and can be mass-produced by techniques that were developed in the microcircuit industry.

ELECTRODE DESIGN

The photolithographic process can be used only on planar surfaces, so that the electrode produced has all the array elements on one plane. In order to accommodate the stimulation tips and feedlines within the limit of 0.50 mm width, the electrode shown in Fig 1 was designed. It is evident that the width of the electrode tips has to be severely restricted if all the feedlines have to pass by them. At the same time the surface area of the stimulating tips must be large enough to ensure that the current density limits for stimulation[5] are not exceeded. The tips can be made longer but then the area of the cochlea stimulated by one tip will overlap with other tips, making it difficult to stimulate only a discrete population of targeted neurons. Moreover, attempts to insert a planar electrode into a chinchilla cochlea showed that the sharp edges of the electrode cut into the soft tissue. Both of these problems could be solved by rolling the planar electrode into a cylinder. The surface area of the stimulating tips can be increased by a large factor and the sharp edges can be eliminated at the same time.

By changing the orientation of the stimulating tips from the longitudinal to the transverse as shown in Fig 2, the tips become "rings" around the electrode and no special measures need be taken during

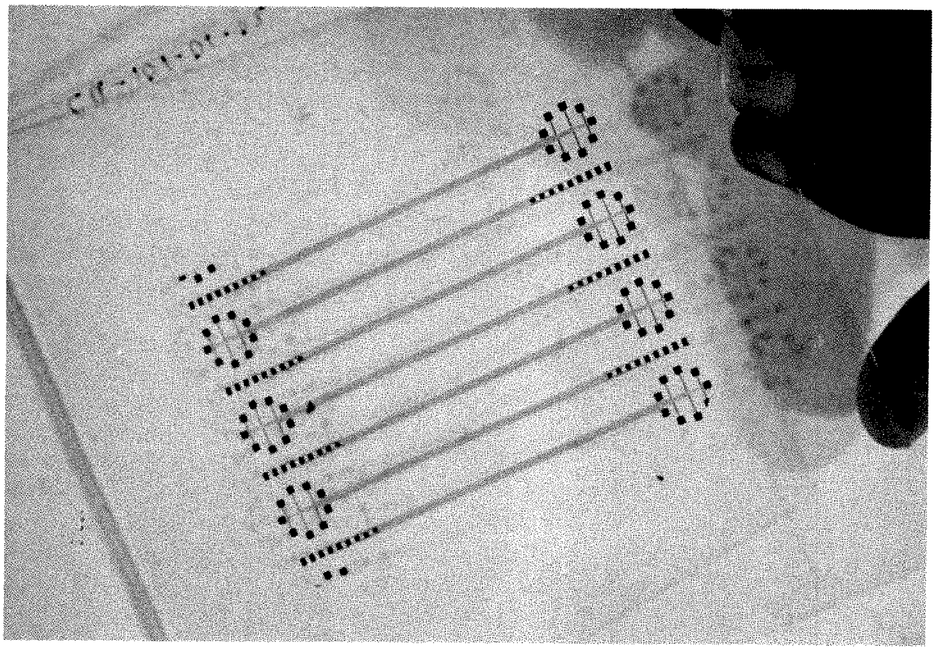

Fig 2. Cylindrical electrode. Mask shows stimulation tips (minimum 0.89 x 1.37 mm, maximum 0.89 x 2.18 mm), feedlines (0.05 mm wide), and connection pads.

the insertion procedure to ensure that the tips are oriented toward the basilar membrane. The technique for rolling the electrode is discussed later.

The masks used for defining the electrode pattern are generated by a computer using a standard software package. It is therefore very easy to change the configuration if this is deemed desirable. The mask design has a number of features that could cause failure under electric and mechanical stress. These include right-angle bends in the track lines and rectangular stimulation pads. The software package that was used could not be adapted easily to produce shapes other than squares and rectangles, and rather than spend the time optimizing the mask design, we placed the emphasis on developing the electrode manufacturing process.

FABRICATION PROCESS

A 10-cm silicon wafer is cleaned and about 1 μm of aluminum is evaporated onto it. A layer of polyimide (PI-2550) 7 μm thick is spun onto the aluminum. This thickness of polyimide is obtained by varying the viscosity of the liquid polymer and the speed of the spinner. The polyimide is then fully cured by baking it in an oven at specified temperatures and periods of time in an atmosphere of nitrogen. A 400-Å layer of titanium followed by a 5,000-Å layer of platinum is deposited in a radio frequency sputtering machine without breaking the vacuum. Positive photoresist is then spun onto the wafer and the parts of the platinum not required for pads, feedlines, or tips are exposed to ultraviolet light. The exposed parts are sputter-etched, leaving the pads, feedlines, and tips. A second layer of 3.5 μm of polyimide is spun onto the structure, and after curing, an aluminum mask is made to protect the whole surface except the polyimide covering the contact pads and stimulating tips. The platinum tips and pads then are exposed by a reactive ion (oxygen) etching process. The wafer then is dipped into a solution of dilute hydrochloric acid that dissolves the top layer of aluminum as well as the layer of aluminum between the silicon wafer and the first layer of polyimide, thus separating the electrode array from the silicon wafer. The array is cut out from the film of polyimide by a specially designed tool that ensures that the sides are straight and the width is 1.571 mm (0.50 x π).

Rolling Planar Electrode Into Cylinder. A specially designed die is used to form the flat electrode into a cylinder. A half-section of the die in shown in Fig 3A. It consists of a 0.50-mm hole with a lead-in funnel. As the planar electrode is pushed into the funnel, it folds over and the edges meet to form a cylinder. The space left in the cylinder is filled with a medical grade silicone rubber, and after curing, the electrode can be removed from the die. A very fine pipette is used to run a line of liquid polyimide along the seam and this is subsequently cured. Figure 3B shows the finished electrode with the transverse bands of platinum taking the form of "rings" around the cylindrical structure.

ELECTRODE TESTING

A number of tests were performed at the stage at which the platinum pattern had been defined on the bottom layer of polyimide but the top layer had not been deposited. The so-called "Scotch tape" test was carried out. There were no signs of lifting of the platinum from the polyimide. The whole structure was placed in a beaker of boiling water for a period of 20 minutes and then examined for delamination. The boiled electrode then was subjected to the Scotch tape test but there was no evidence of

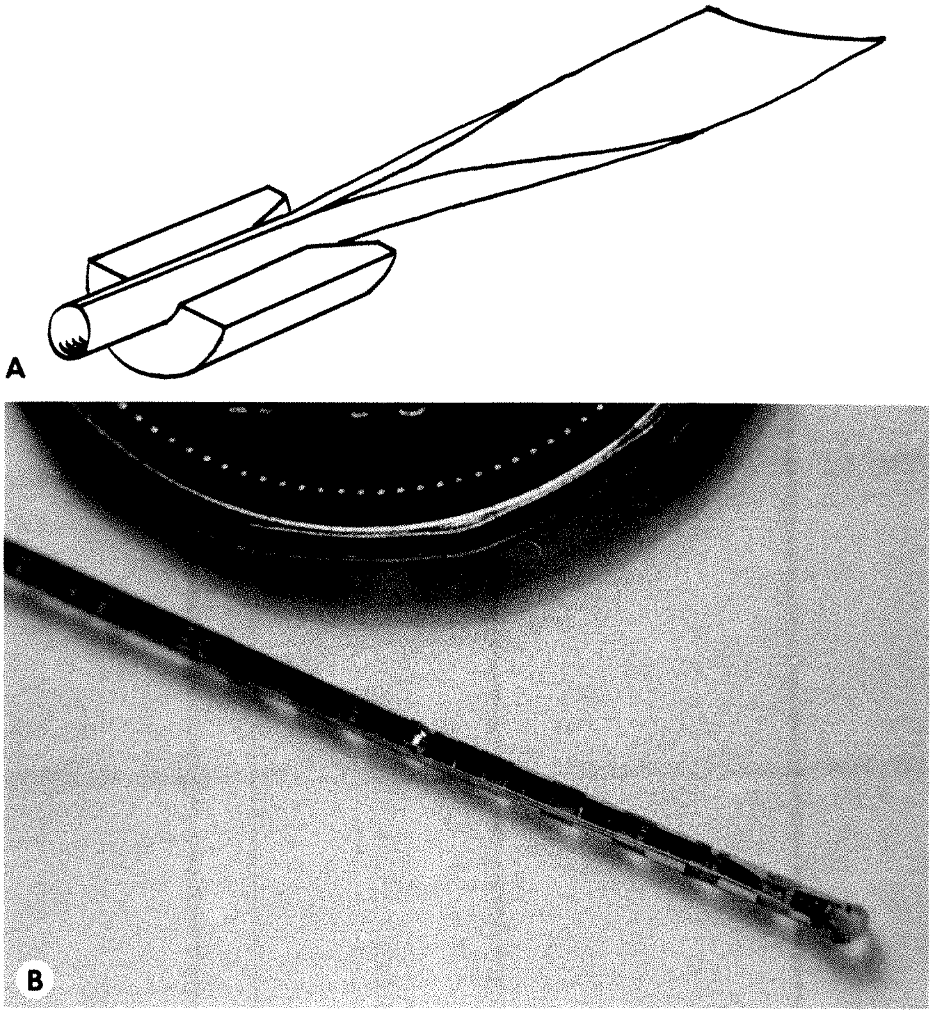

Fig 3. A) Half-section of die used to form electrode into cylinder. B) Finished cylindrical electrode showing stimulation tips as transverse bands of platinum. Grid spacing in background is 6 mm.

delamination. Attempts were made to scrape off the platinum with a knife. The result was that both the platinum and polyimide were torn up.

At the point at which the top layer of polyimide had been etched through to expose the pads and stimulating tip, the Scotch tape test was repeated. The platinum did not separate from the polyimide. The pads were "probed" with a standard microcircuit prober and the probe was drawn across the pads. Delamination was considered to have occurred if the platinum cracked and curled away from the polyimide. If this treatment produced a scratch the electrode was considered to have maintained its integrity.

Several tests are being carried out currently to test the electrode for its suitability for use in humans. The electrode has been inserted and withdrawn more than 50 times from a clear plastic "cochlea" that was molded from a cadaver temporal bone. The electrode then was examined for damage and none was found. A second test consists of wrapping the electrode around a brass cone machined to dimensions taken from a human temporal bone. The third stimulating tip from the apical end cracked during this test. This test puts a very severe stress on the platinum tip, which is already bent to form a cylinder and then forced to bend along the axis of the cylinder. However, it is interesting that neither the first nor the second stimulating tip cracked even though they were forced to follow a curve whose radius of curvature

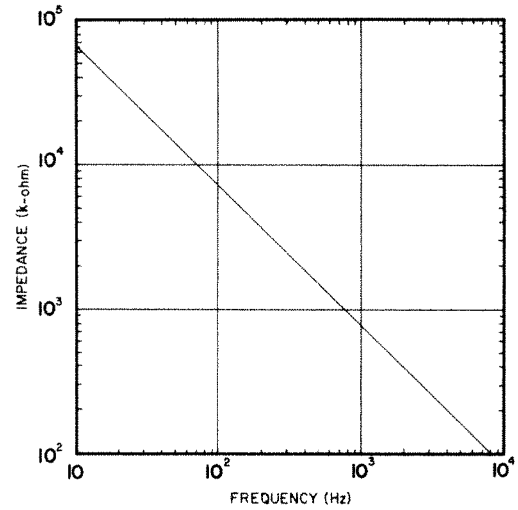

Fig 4. Impedance of electrode immersed in artificial perilymph at current density of 100 mA/cm².

COMPARISON WITH OTHER ELECTRODES

	Vienna	*Nucleus*	*Cylindrical*
Stimulating tip area (mm^2)	0.06	0.5	1.2
Stimulating tip structure	Part sphere	Ring	Ring
No. in array	16	22	9
Impedance at 1 kHz (kΩ)	3.5	(Not measured)	1.0
Manufacturing method	Hand	Hand	Photolithographic
Conductor material	Platinum	Platinum	Platinum (sputtered)
Insulator material	Silicone rubber	Silicone rubber	Polyimide/silicone rubber

was less than the third one. This particular test has to be repeated with a large enough sample to give meaningful results.

The electrode shortly will be inserted into real cadaver cochleas and sections will be taken for examination under a microscope to determine what, if any, damage the electrode will do to the cochleas.

The cylindrical electrode has not been tested for toxicity since it is unsuitable for insertion into a chinchilla cochlea. However, small planar electrodes made from the same materials by the same process have been tested extensively in chinchillas over periods ranging from a few weeks to over 1 year. So far, no adverse effects of the electrode have been found.

A batch of electrodes currently are undergoing in vitro tests in a bath of artificial perilymph at room temperature. A biphasic pulse with a current density of 100 mA/cm^2, a pulse rate of 300 Hz, and a pulse width of 0.3 ms is used in these tests. The electrodes are removed, inspected visually, photographed, and tested for deterioration of the insulating material at 2-week intervals. So far no deterioration of the platinum tips or the polyimide has been detected in the 12 months since the tests started. In the last 3 months, the current density was increased to a level corresponding to the nongassing limit of pure platinum[5] on two of the electrodes. These show a slight change in color in comparison to the others that might indicate some corrosion on the surface. There were, however, no signs of delamination. An accelerated lifetime test at 80°C with maximum nongassing current stimulation is in progress.

Figure 4 shows the impedance of the electrode in artificial perilymph as a function of frequency. The relatively low impedance makes it attractive for use in conjunction with low voltage complementary metal oxide semiconductor circuits.

The results obtained so far look promising. However, more data need to be collected on the performance of the electrode to ensure that it will meet all the requirements, which are sometimes contradictory. This process requires time and perhaps several changes in the design.

COMPARISON WITH OTHER ELECTRODES

The Table presents a comparison of the cylindrical electrode with the Vienna and Nucleus electrodes.

CONCLUSION

A flexible scala tympani electrode has been developed by standard photolithographic techniques. The stimulating tips, feedlines, and pads are made of platinum and this is deposited and etched by radio frequency sputtering. The substrate material is a polyimide film, which is deposited in liquid form by spinning followed by a high-temperature curing process. The masks for defining the electrode layout are generated by a standard computer software package. Except for the use of dilute hydrochloric acid (which is present in perilymph), all other etchants that may be toxic to living tissue are avoided.

The mechanical deformation properties of the electrode array can be adjusted by varying the thickness of the polyimide film. Its buckling characteristics can be improved by inserting a fine stainless steel wire in the silicone rubber.

In electric tests, currents above expected stimulation requirements have been passed through the electrode but no deterioration of the platinum or the polyimide has been observed so far. In tests in which the current density was raised to the nongassing limit of pure platinum, some change of color of the platinum has been noted. The impedance of the electrode at 1 kHz is about 1 kΩ and therefore low voltage complementary metal oxide semiconductor circuitry can be used to drive it.

Tests for toxicity continue in chinchillas and insertion tests using cadaver temporal bones will be carried out shortly.

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BIOCHEMICAL STUDY OF PROTEASE INHIBITORS IN NORMAL CHINCHILLA MIDDLE EAR

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Protein concentration and inhibitory capacity of both α1-antitrypsin (α1-AT) and α2-macroglobulin (α2-M) were measured in plasma and middle ear bulla (MEB) washings of chinchillas by use of specific antisera against chinchilla α1-AT and α2-M. Low molecular weight (LMW) trypsin inhibitor also was analyzed in MEB washings. Chinchilla α2-M showed a common antigenicity with human α2-M. The mean value of α1-AT in chinchilla plasma was 412.0 ± 87.8 and that of α2-M was 435.0 ± 117.1 mg/dL. There was a significant relationship between α1-AT level and antitryptic activity, and between α2-M level and trypsin binding activity in plasma. The majority of α1-AT and α2-M in plasma is present as free inhibitors unsaturated with proteases. The MEB washings had significant antitryptic activity, which is attributed to both α1-AT and LMW trypsin inhibitors. Inhibitory functions of α1-AT and LMW trypsin inhibitors appear to play an important role in the defense of the normal middle ear mucosa.

KEY WORDS — α1-antitrypsin, α2-macroglobulin, antitryptic activity, low molecular weight trypsin inhibitor, middle ear bulla washing, trypsin binding activity.

INTRODUCTION

Protease inhibitors occupy the largest portion of plasma proteins except for albumin and consist of at least eight different inhibitors: α1 (α1-antitrypsin [AT], α1-antichymotrypsin, inter-α-trypsin inhibitor), α2 (α2-macroglobulin [M], α2-plasmin inhibitor), and others (antithrombin III, C1-inactivator, and protein C inhibitor).[1]

They play important roles not only in controlling the physiologic levels of tissue protease activity,[2] the coagulation-fibrinolysis system,[3] and protection of normal mucosal tissues,[4] but also in the regulation of bacterial and lysosomal protease activity during inflammation.[4] Together, α1-AT and α2-M constitute 90% of the inhibitory function in plasma, and α1-AT is well known as an acute phase reactant,[5] an excellent index for reflecting the severity of systemic inflammatory change.

The chinchilla (*Chinchilla laniger*) has been used widely in recent years as a laboratory animal for experimental otitis media.[6,7]

Otitis media with effusion (OME) is the most prevalent middle ear disease. In recent years, the protease-inhibitor system in human OME has been investigated.[8-10] However, protease inhibitors in the normal middle ear and their kinetics during the course of OME have not been characterized adequately. In order to clarify the significance of protease inhibitor in the normal middle ear, the protein concentration and inhibitory capacity of both α1-AT and α2-M in the middle ear bulla (MEB) washings of normal chinchillas were measured and compared to those in bronchial lavage fluids (BLF). The presence of low molecular weight (LMW) trypsin inhibitors was also examined in the MEB washings.

MATERIALS AND METHODS

MATERIALS

Chinchilla blood was obtained by cardiac puncture from 30 animals under anesthesia with ketamine HCl (20 mg/kg, intramuscularly) and collected into a tube containing 3.8% sodium citrate (v/v; 0.1:1). After centrifugation at 2,500 rpm for 20 minutes, plasma was recovered and stored at −80°C until use. Ten normal chinchillas weighing between 400 and 600 g were anesthetized with ketamine HCl. After 1.5 mL of sterile saline was directly injected into both sides of the chinchilla MEB, the lavage fluid retained in the MEB was recovered (about 70% to 80% of the original volume of the saline could be recovered). Any recovered fluid contaminated with blood macroscopically was excluded from this study. After centrifugation at 2,500 rpm for 10 minutes, the supernatants were recovered and stored at −80°C until use. Immediately after painless killing of the ten chinchillas by cardiac puncture, the anterior wall of the trachea was cut off to insert the small plastic catheter into

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REPRINTS — Yukiyoshi Hamaguchi, MD, Dept of Otorhinolaryngology, Mie University School of Medicine, 2-174 Edobashi, Tsu, Mie, 514, Japan.

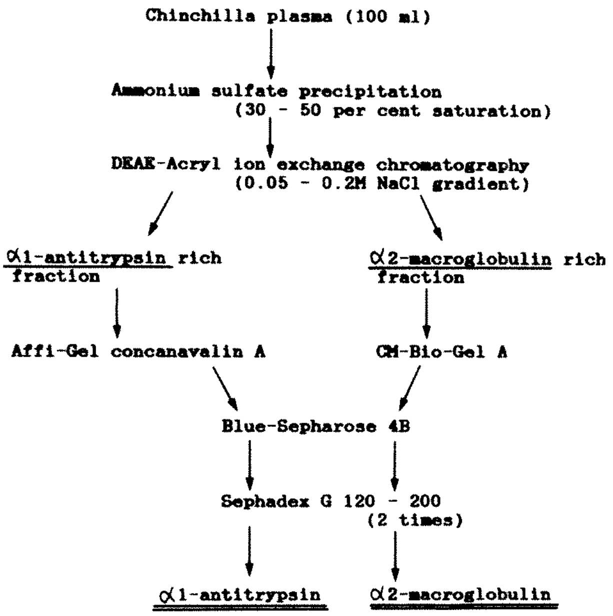
Chinchilla plasma (100 ml)
Ammonium sulfate precipitation
(30 - 50 per cent saturation)
DEAE-Acryl ion exchange chromatography
(0.05 - 0.2M NaCl gradient)
α1-antitrypsin rich fraction
α2-macroglobulin rich fraction
Affi-Gel concanavalin A
CM-Bio-Gel A
Blue-Sepharose 4B
Sephadex G 120 - 200
(2 times)
α1-antitrypsin
α2-macroglobulin

Fig 1. Purification procedure of α1-antitrypsin and α2-macroglobulin from chinchilla plasma.

the trachea. Two milliliters of sterile saline were injected slowly through the catheter and recovered into the syringe under slightly negative pressure. This procedure was repeated three times, then BLF was recovered into the syringe. After centrifugation at 2,500 rpm for 10 minutes, the supernatants were recovered and stored at −80°C.

PURIFICATION OF CHINCHILLA α1-AT AND α2-M

α1-Antitrypsin was purified by a modification of the method of Musiani and Tomasi,[11] and α2-M by a modification of the method of Nagasawa et al.[12] All the procedures are summarized in Fig 1.

Ammonium Sulfate Saturation. Two milliliters of 10% dextran sulfate and 10 mL of 1 *M* CaCl2 were mixed with 100 mL of chinchilla plasma to remove low density lipoproteins. The precipitant formed was removed by centrifugation, and the supernatant was dialyzed overnight against 0.02 *M* sodium phosphate buffer (PB; pH 8.0). To the supernatant saturated ammonium sulfate was added to make 30% saturation under stirring. After centrifugation at 1,500 rpm for 15 minutes, the supernatant was brought to 50% saturation with saturated ammonium sulfate solution. The pseudoglobulin fraction precipitated was collected by centrifugation at 3,500 rpm for 30 minutes and dialyzed three times against 1 L of 0.05 *M* Tris-HCl (pH 8.8) containing 0.05 *M* NaCl for 48 hours.

DEAE-Acryl Ion Exchange Chromatography. The dialyzed fraction was applied to a column of DEAE-Acryl (2.5 x 30 cm; LKB, Stockholm) equilibrated with 0.05 *M* Tris-HCl (pH 8.8) containing 0.05 *M* NaCl (buffer A). After washing the column with 150 mL of buffer A, the elution was developed by a linear gradient with 0.05 *M* NaCl to 0.2 *M* NaCl in 0.05 *M* Tris-HCl buffer. The α1-AT–rich fraction and α2-M–rich fraction were pooled respectively, the former was dialyzed against 0.01 *M* PB (pH 7.4) containing 0.5 *M* NaCl overnight, and the latter was dialyzed against 0.01 *M* acetate buffer (pH 6.0) for 48 hours.

Affi-Gel Con A and CM-Bio-Gel A Chromatographies. Dialyzed α1-AT–rich fraction was applied to Affi-Gel Con A (1.5 x 15 cm; Bio Rad, Richmond) equilibrated with 0.01 *M* PB (pH 7.4) containing 0.5 *M* NaCl. After unbound protein was eluted with the buffer, the α1-AT was eluted with the buffer containing 0.1 *M* α-D-methylglucopyranoside and dialyzed against the buffer overnight. The dialyzed α2-M–rich fraction was applied to a column of CM-Bio-Gel A (2.5 x 30 cm; Bio Rad) equilibrated with 0.01 *M* acetate buffer (pH 6.0). The α2-M was recovered in the unbound fraction and dialyzed against 0.01 *M* PB (pH 7.4) containing 0.5 *M* NaCl.

Blue-Sepharose 4B Chromatography. Both α1-AT– and α2-M–rich fractions were applied to a column of Blue-Sepharose 4B (1.5 x 15 cm; Pharmacia Fine Chemicals, Uppsala, Sweden) equilibrated with 0.01 *M* PB (pH 7.4) containing 0.5 *M* NaCl. Both inhibitors were recovered in the unbound fractions and dialyzed against 0.01 *M* PB (pH 7.4), then concentrated about ten times with Sephadex G-75 powder.

Sephadex G-120-200 Gel Filtration. Concentrated α1-AT– and α2-M–rich fractions were applied two times to a column of Sephadex G-120-200 (1.5 x 100 cm; Sigma) equilibrated with 0.01 *M* PB. The α1-AT– and α2-M–rich fractions were pooled and concentrated.

IMMUNIZATION OF CHINCHILLA α1-AT AND α2-M AND PREPARATION OF ANTIBODIES

New Zealand white rabbits were immunized with 1 mg of purified α1-AT or α2-M mixed 1:1 with complete Freund's adjuvant. The emulsion was injected subcutaneously under the back skin. Then booster injections (the same amount of purified inhibitor) were given at 2 and 6 weeks after the first injection. Circulating antibody titers were expressed as the maximum dilution ratio (times 2^x) of serum that made a precipitin line against 1 mg/mL of purified inhibitor by the Ouchterlony[13] method. When antibody titers reached between times 2^5 and times 2^6, a blood sample was collected by cardiac puncture. After centrifugation at 3,000 rpm for 20 minutes at 4°C, clear serum was recovered and used as specific antibody for the inhibitor assay.

QUALITATIVE AND ELECTROPHORETIC ANALYSES

Determination of α1-AT in the fractions was made by measuring antitryptic activity (ATA) in each fraction, and that of α2-M was by the Ouchterlony method using a commercial human

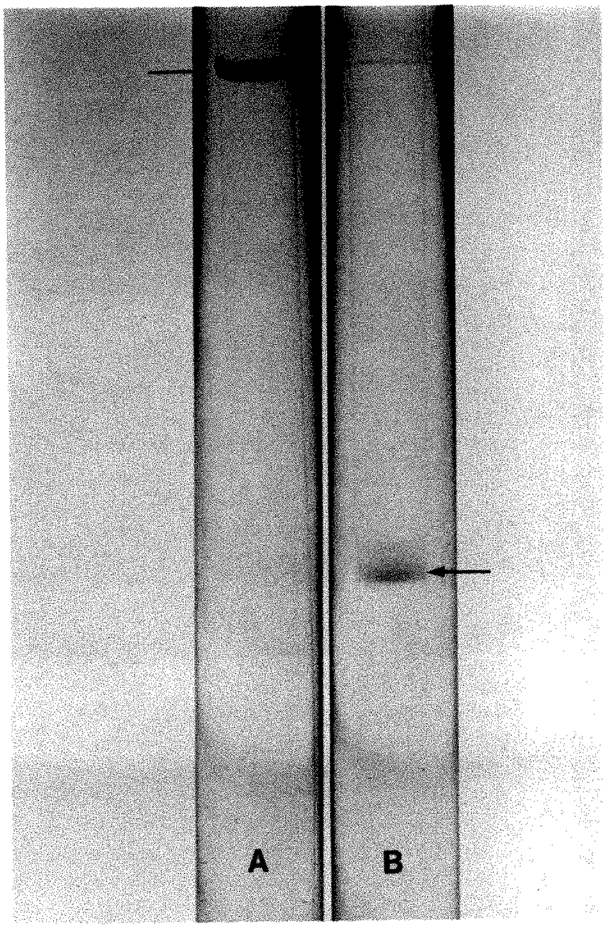

Fig 2. Ten percent SDS-polyacrylamide gel electrophoresis of purified chinchilla A) α2-M and B) α1-AT. Single protein band (indicated by arrows) could be observed in each purified inhibitor.

α2-M antibody. Analytic 10% SDS-polyacrylamide gel electrophoresis was performed according to the method of Laemmli.[14] Specificity of each inhibitor was analyzed by immunoelectrophoresis.

ASSAY FOR AMIDOLYTIC ACTIVITY

Amidolytic activity was measured by using a specific substrate for trypsin, benzoyl-L-arginine-*p*-nitroanilide (BAPNA, Sigma), according to the method of Eriksson.[15] Fifty microliters of the sample were incubated with 1 m*M* of BAPNA dissolved with 0.1 *M* Tris-HCl (pH 8.2) containing 0.02 *M* $CaCl_2$. After incubation at 25°C for 20 minutes, the reaction was stopped by adding 0.5 mL of 30% acetic acid. The absorbance of the product was read at 410 nm. Amidolytic activity was expressed as the difference of the absorbance between each sample and a control.

ASSAY FOR ANTITRYPTIC ACTIVITY

The functional capacity of α1-AT to inhibit tryptic (amidolytic) activity was analyzed by measuring the inhibition of hydrolysis of the specific substrate BAPNA. Fifty microliters of the sample were incubated with the same volume of bovine pancreas trypsin (1.25 mg/mL; type III, Sigma) at 37°C for 20 minutes, then 1 m*M* of BAPNA dissolved with 0.1 *M* Tris-HCl (pH 8.2) containing 0.02 *M* $CaCl_2$ was added to the mixture. After incubation at 25°C for 20 minutes, the residual amidolytic activity was measured by the procedure described above. The level of ATA was expressed in the percent of inhibition against hydrolytic activity in the control, which did not contain α1-AT.

ASSAY FOR TRYPSIN BINDING ACTIVITY

The functional capacity of α2-M to form a complex with trypsin was assessed by measuring the residual amidolytic activity of a mixture of α2-M and trypsin against the substrate (BAPNA) after the addition of soybean trypsin inhibitor (SBTI).[16] After incubation of the sample with trypsin, excess amounts of SBTI (2 mg/mL; Sigma) were added to the mixture to inhibit free trypsin. The mixture was incubated at 25°C for 20 minutes, and the residual amidolytic activity was measured by the procedure described above. The level of trypsin binding activity (TBA) was expressed as the difference of the absorbance at 410 nm between each sample and the control with no α2-M.

ASSAY FOR α1-AT AND α2-M LEVELS

The α1-AT level was measured by single radial immunodiffusion,[17] and that of α2-M by electroimmunodiffusion.[18]

ANALYSIS OF LMW TRYPSIN INHIBITORS

Pooled MEB washings were concentrated to 1.5 mg/mL (0.5 mL), then applied to a column of Sephadex G-75 (1.5 x 30 cm; Pharmacia) equilibrated with 0.1 *M* PB, pH 7.4. The concentration of α1-AT and the ATA were measured in each fraction.

ASSAY FOR TOTAL PROTEIN CONCENTRATION

Total protein concentration (TPC) was measured by spectrophotometry using a commercial kit (Biuret reagent from Sigma Diagnostics).

RESULTS

The purified α1-AT and α2-M gave a single protein band upon 10% SDS-polyacrylamide gel electrophoresis (Fig 2). On immunoelectrophoretic examination of purified α1-AT and α2-M, a single sharp precipitin line located at α1 and α2 globulin regions was developed against anti-whole chinchilla serum (Fig 3). The results of cross reactivity between human and chinchilla antisera against purified α1-AT and α2-M are shown in Fig 4. Purified human α2-M gave a precipitin line against anti-chinchilla α2-M, and purified chinchilla α2-M gave a precipitin line against anti-human α2-M. However, purified human α1-AT gave a faint precipitin line against anti-chinchilla α1-AT, and purified chinchilla α1-AT gave no line against anti-human α1-AT antibody.

ATA of Purified Chinchilla α1-AT and α2-M. The results are summarized in Fig 5A. Purified

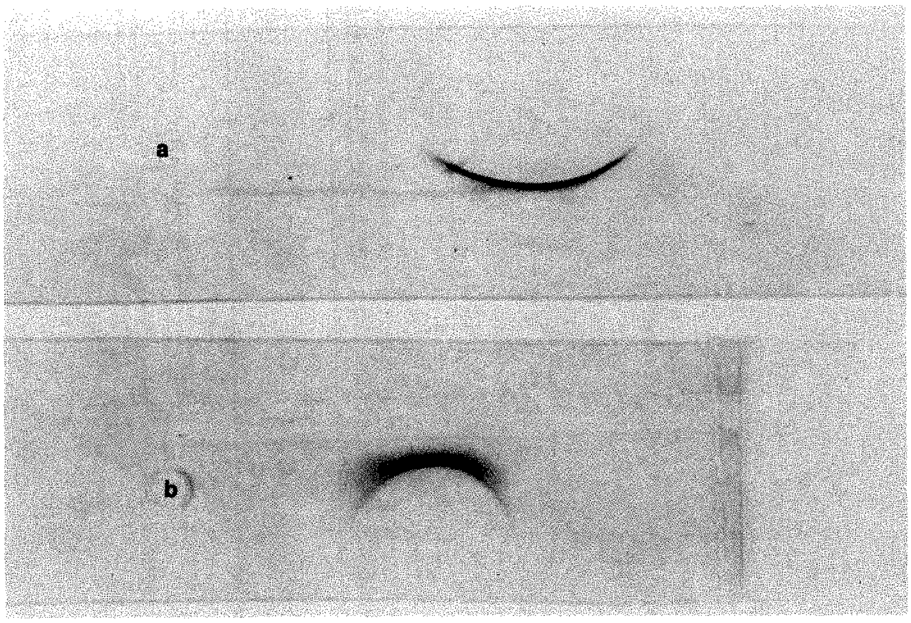

Fig 3. Immunoelectrophoresis of purified chinchilla α1- AT (a) and α2-M (b) against rabbit anti-whole chinchilla serum. Single precipitin line was developed in each purified inhibitor at α1 and α2 globulin region against anti-whole chinchilla serum.

α1-AT (nondiluted α1-AT had about 35 μg of α1-AT) inhibited the amidolytic activity of bovine trypsin in proportion to its dilution ratio. However, purified α2-M showed very low ATA in this assay system.

TBA of Purified Chinchilla α1-AT and α2-M. Purified α2-M (nondiluted α2-M had about 20 μg of α2-M) exhibited residual amidolytic activity in proportion to its dilution radio. However, α1-AT had no amidolytic activity (Fig 5B).

Analysis of α1-AT and α2-M in MEB Washings. Both amidolytic (tryptic) activity and ATA are shown in Fig 6. Pooled BLF had amidolytic activity about three times higher than pooled MEB washings, although the TPC of the pooled BLF (230 mg/dL) was about four times higher than the concentrated MEB washings (55 mg/dL, Fig 6A). On the other hand, MEB washings had a significantly higher ATA than BLF ($p < .01$, Fig 6B). Both concentrated MEB washings and BLF gave precipitin lines against anti-α1-AT antibody, while no line could be detected against anti-α2-M antibody. The ATA and TBA in each MEB washing ($n = 16$) are shown in Fig 7. Mean value of ATA was 32.9% ± 4.49% and no TBA could be detected in any of the 16 samples. Amidolytic activity was weak (under 0.04 optical density level) in all the MEB washing samples.

Analysis of LMW Trypsin Inhibitors in MEB Washings. The elution profile of Sephadex G-75 is shown in Fig 8. Two major peaks of ATA could be detected, and the peak near void volume (Vo) had a precipitin line against anti-α1-AT antibody. The peak in the lower molecular weight fractions had a level of ATA similar to the first peak, but a much lower TPC.

Relationship Between α1-AT Level and ATA, and Between α2-M Level and TBA. The mean value of α1-AT level in normal chinchilla plasma was 412.0 ± 87.8 mg/dL and that of α2-M was 435.0 ± 117.1 mg/dL. A significant correlation between α1-AT level and ATA could be detected ($n = 20$, $r = 0.67$, $p < .01$), and one could also be detected between α2-M level and TBA ($n = 21$, $r = 0.81$, $p < .01$) (Fig 9).

DISCUSSION

The results of cross reactivity between human and chinchilla inhibitors indicate that chinchilla

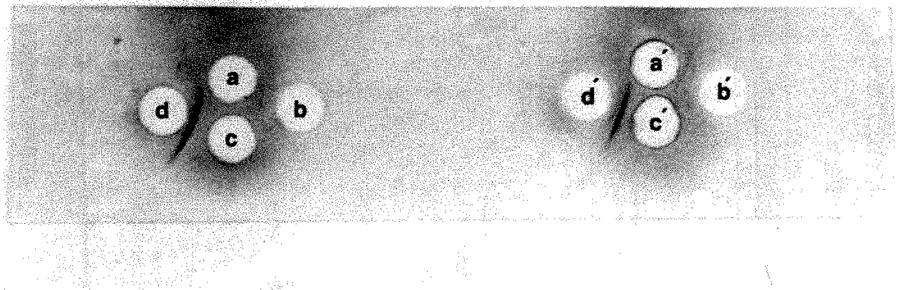

Fig 4. Ouchterlony analysis of cross reactivity between human and chinchilla antisera against α1-AT and α2-M. Single precipitin line could be observed between c and d, and between c′ and d′. a — anti-human α1-AT, b — purified chinchilla α1-AT, c — anti-human α2-M, d — purified chinchilla α2-M, a′ — anti-chinchilla α1-AT, b′ — purified human α1-AT, c′ — anti-chinchilla α2-M, d′ — purified human α2-M.

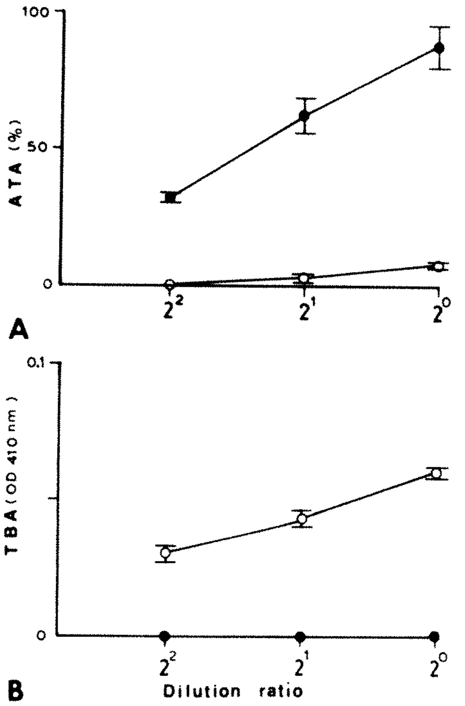

Fig 5. A) Antitryptic activity (ATA; mean ± SD) of purified chinchilla α1-AT (dark circles) and α2-M (light circles) plotted against dilution ratios of each inhibitor. ATA of α1-AT was proportional to its level (dilution ratio), while α2-M had very low ATA. B) Trypsin binding activity (TBA; mean ± SD) of purified chinchilla α1-AT (dark circles) and α2-M (light circles) expressed in optical density (OD) at 410 nm, plotted against dilution ratio of each inhibitor. TBA of α2-M was proportional to its level (dilution ratio), while α1-AT had no TBA.

α2-M has a common antigenicity with human α2-M, but chinchilla α1-AT has little common antigenicity with human α1-AT. Previous study has shown the common antigenicity of α2-M between humans and different animal species.[19] Therefore, it may be possible to measure the α2-M level in chinchilla plasma and other body fluids by using a commercial anti-human α2-M antibody.

The results of the ATA assay indicate that the inhibition mechanism against trypsin is different between α1-AT and α2-M. As α1-AT can combine with trypsin to inhibit its hydrolytic activity against the specific substrate (BAPNA), the level of inhibition is proportional to the α1-AT level measured by specific anti-α1-AT serum (Fig 5A). On the other hand, α2-M can combine with trypsin, leaving the active site intact. The α2-M–trypsin complex has residual tryptic activity against LMW substrates such as BAPNA.[20] The residual activity in the presence of excess SBTI represents the TBA of α2-M (Fig 5B).

Both α1-AT and α2-M inhibit protease activity by forming protease-inhibitor complexes, and inhibitors consist of both free and complexed inhibitors. Both α1-AT and α2-M levels measured by immunochemical assay reflect total (free and complexed) inhibitor levels, while the ATA and the TBA reflect free inhibitor levels. In the present study, chinchilla plasma had high ATA and TBA similar to those of human plasma,[15] and significant correlations could

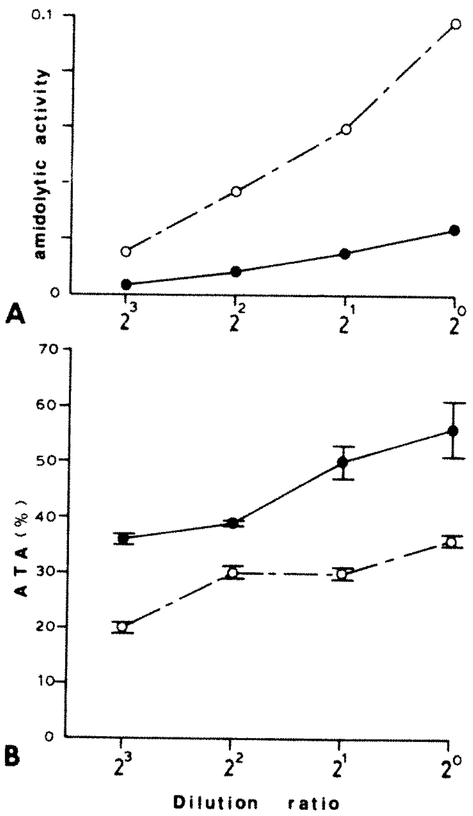

Fig 6. A) Amidolytic activity (mean) and B) antitryptic activity (ATA; mean ± SD) in pooled chinchilla middle ear bulla (MEB) washings (dark circles) and bronchial lavage fluid (BLF; light circles), plotted against dilution ratios. Total protein concentration of nondiluted MEB washings was 55 mg/dL, and that of BLF was 230 mg/dL. Value of ATA in MEB washings was significantly higher than that in BLF (p<.01), while mean value of amidolytic activity in BLF was about three times higher than that in MEB washings.

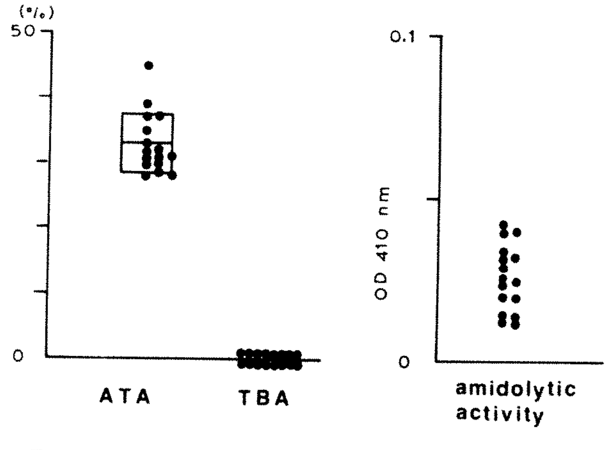

Fig 7. Amidolytic activity, antitryptic activity (ATA), and trypsin binding activity (TBA) in each middle ear bulla (MEB) washing (n = 16). No TBA was detected in any MEB washing, and amidolytic activity was very low in all samples (under 0.04 optical density [OD]).

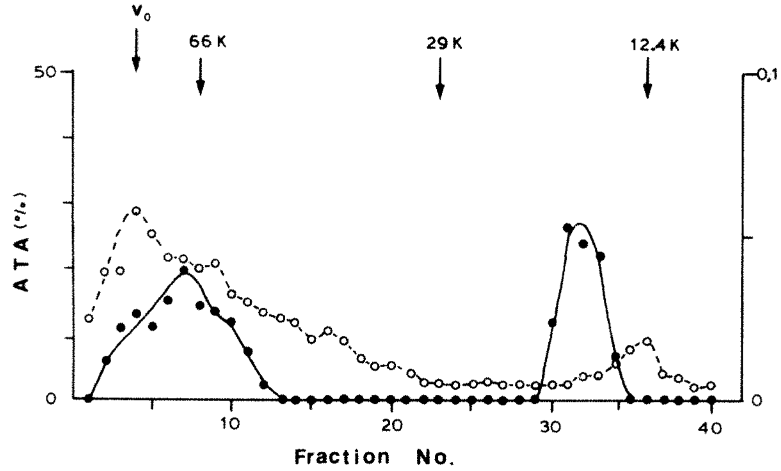

Fig 8. Elution profile of Sephadex G-75 in pooled middle ear bulla washings concentrated to 1.5 mg/mL by use of 0.1 *M* phosphate buffer (pH 7.4), 20 mL/h flow rate, and 0.5 mL fraction size. Two major peaks of antitryptic activity (ATA; dark circles) could be detected, and first peak, near void volume (Vo), had precipitin line against anti αl-AT antibody, while later peak at lower molecular weight had no precipitin line. Light circles — optical density (OD).

be observed between αl-AT level and the ATA, and between α2-M level and the TBA, in chinchilla plasma. These results suggest that the bulk of both inhibitors in plasma is occupied by free, unsaturated inhibitors.

In the present study, MEB washing fluids were used as samples. Since it is very difficult to collect substances on the mucosal surface of the normal middle ear mucosa (MEM), saline lavage seems to be a good method for sampling.

Analysis of MEB washings by Sephadex G-75 indicates that MEB washings contain two different trypsin inhibitors: αl-AT and LMW trypsin inhibitors. They may be secreted and/or leaked from the MEM into the mucosal surface layer, and have significant ATA. These inhibitors have an important role in the regulation of protease activity from various sources (leukocytes, bacteria, etc), protecting bronchial mucosae in both normal and inflammatory conditions.[21] Therefore, ATA in MEB washings may contribute to the defense mechanism in the MEM against proteolytic invasions such as bacterial proteases. In the elution profile of Sephadex G-75, LMW trypsin inhibitors had similar levels of ATA to αl-AT eluting near Vo, suggesting the importance of LMW inhibitors in the protection of normal MEM. The absence of TBA in the MEB washings means that α2-M is not present in the normal MEM surface, possibly because it has difficulty leaking from the vessels because of its high molecular weight. In the normal condition, α2-M seems to play a role as an intravascular inhibitor.[2]

In a human OME study, LMW inhibitors were detected in MEEs and MEM with chronic OME, and found to be produced in the serous glands of the MEM.[22] In other external secretions such as nasal secretions,[23] bronchial sputum,[24] and urine,[25] LMW inhibitors already have been purified and characterized by many investigators. Orlowski et al[21] reported that LMW inhibitors in bronchial sputum would derive from the split products of inter-α-

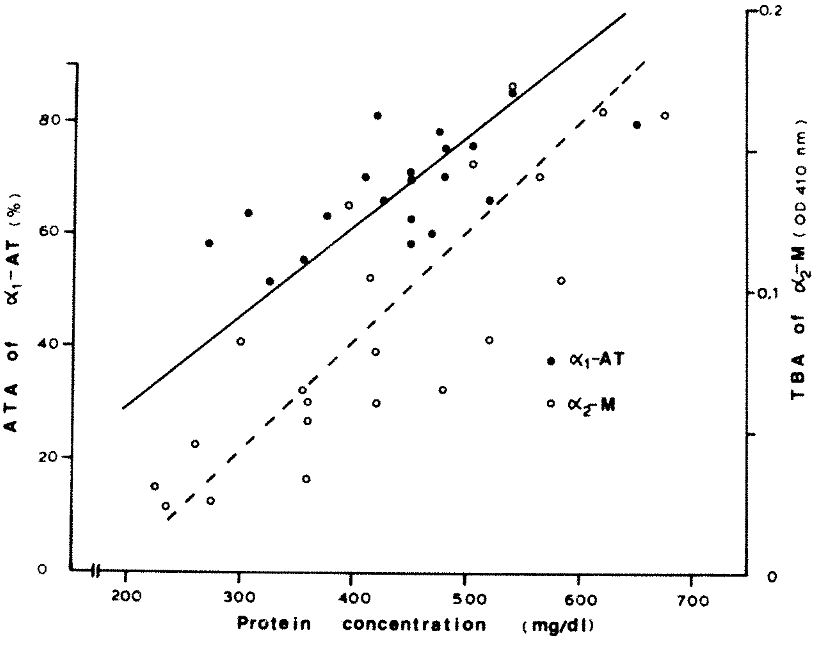

Fig 9. Relationships between αl-AT level and ATA and between α2-M level and TBA in chinchilla plasma. Significant correlation could be detected between αl-AT level and ATA (n = 20, r = 0.67, p < .01), and between α2-M level and TBA (n = 21, r = 0.81, p < .01).

trypsin inhibitor in plasma. Bronchial lavage fluid had higher amidolytic activity and lower ATA than MEB washings, suggesting that the balance of the protease-inhibitor system would be different between normal bronchial mucosa and MEM. In general, BLF contains considerable amounts of alveolar macrophages and other cells. Therefore, the higher amidolytic activity in BLF than MEB washings might be attributed to lysosomal proteases, especially α1-AT- and LMW inhibitor-resistant thiol proteases in BLF that are released from alveolar macrophages.[26] Since bronchial mucosa has more exposure to bacterial infections than the MEM because of its anatomic situation, it would need a much stronger protective system. The difference in ATA and amidolytic activity between BLF and MEB washings may reflect the difference between the defense systems in the two mucosae.

It is concluded that 1) chinchilla α2-M has common antigenicity with human α2-M; 2) the mean value of α1-AT in chinchilla plasma was 412.0 ± 87.8 mg/dL, and that of α2-M was 435.0 ± 117.1 mg/dL; 3) the majority of α1-AT or α2-M in plasma is shown to be present as free inhibitor unsaturated with proteases; 4) MEB washings showed considerably high ATA, which was attributed to both α1-AT and LMW inhibitors; and 5) these inhibitors appear to play an important role in the defense system of the MEM.

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MUCOCILIARY DISEASE OF THE MIDDLE EAR DURING EXPERIMENTAL OTITIS MEDIA WITH EFFUSION INDUCED BY BACTERIAL ENDOTOXIN

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Lipopolysaccharide (10 μg/mL) derived from *Klebsiella pneumoniae* was injected into the middle ear of guinea pigs. The animals were killed painlessly on days 1, 3, and 7 after inoculation, and the mucosal samples from two sites within the tympanic cavity, close to the tympanic orifice and distal to the orifice, were examined for ciliary activity and epithelial morphology. At day 1 and day 3 serous effusion was observed and deterioration of ciliary activity and morphologic changes were observed. No effusion was recognized at day 7, when the ciliary activity in the distal mucosa was still diminished and that in the proximal mucosa had recovered to a normal level. Our data have shown that lipopolysaccharide extracted from *K pneumoniae* can produce otitis media with effusion in laboratory animals, and dysfunction of cilia due to lipopolysaccharide probably is responsible for the accumulation of middle ear effusion. The mucociliary system is indeed an important defense system and failure of such a system, especially in the mucosa close to the tympanic orifice, can cause the buildup of effusions.

KEY WORDS — bacterial endotoxin, ciliary activity, electron microscopy, otitis media with effusion.

INTRODUCTION

Otitis media with effusion (OME), or secretory otitis media, has previously been considered a noninfectious condition caused by tubal obstruction or allergy. However, since Senturia et al[1] demonstrated that 42% of OME cases have culturable bacteria, much new evidence has accumulated to the effect that bacterial involvement may be one of the major responsible pathogenetic factors in the manifestation and development of OME. Using the limulus amebocyte lysate assay, Bernstein et al[2] demonstrated that middle ear effusions (MEEs) from three of 17 cases contained endotoxin. Using a similar assay, DeMaria et al[3] examined MEEs from 89 children with chronic otitis media. They reported that 71 (81%) of the samples exhibited endotoxin activity in concentrations ranging from 0.5 to more than 512 ng/mL and endotoxin was also present in 67% of the culture-negative MEEs. These reports on the discovery of endotoxin in MEEs from patients with chronic OME suggest its likely involvement in the pathogenesis of the condition.

Recent experimental studies have argued strongly in favor of the probable role of *Hemophilus influenzae* endotoxin in the origin and development of OME.[4,5] DeMaria et al[4] have observed clear, amber-colored MEEs in chinchillas after inoculation of killed *H influenzae* organisms or endotoxin purified from these bacteria into the tympanic cavity. Nonomura et al[5] also have observed massive MEEs in the guinea pig inoculated with 10 μg/mL of lipopolysaccharide (LPS) extracted from *H influenzae* type b. They presented us with the concept that direct inoculation of endotoxin or LPS derived from *H influenzae* is a successful way to create OME in laboratory animals and that endotoxin may be involved in the clinical development of the disease. Although it is well proven that capillary injury and increased vascular permeability due to endotoxin are the probable mechanisms for MEE production in their models,[4,5] clarification still is needed for the exact mechanism responsible for failure of MEE to be cleared via the eustachian tube, resulting in the accumulation of fluid in the tympanic cavity. If the mucociliary system of the tubotympanum is functioning effectively, any effusion formed must be removed to the pharynx. In our previous study[6] we reported that direct inoculation of *Klebsiella pneumoniae* LPS can induce serous OME in the guinea pig and that the mucociliary system of the eustachian tube is affected in such a condition.

The density of ciliated cells is not constant in the tubotympanum.[7,8] The beating activity of ciliated cells differs according to locations within the tubotympanum,[9,10] and the ciliary activity displays a varying pattern of reactions to pathologic agents according to the locations.[11-15] Thus, we have examined the ciliary activity within the tympanic cavity during an experimental OME due to direct inoculation of *K pneumoniae* LPS, with special reference to the varying reactions of ciliated cells according to their locations.

MATERIALS AND METHODS

Thirty Hartley strain male guinea pigs (average

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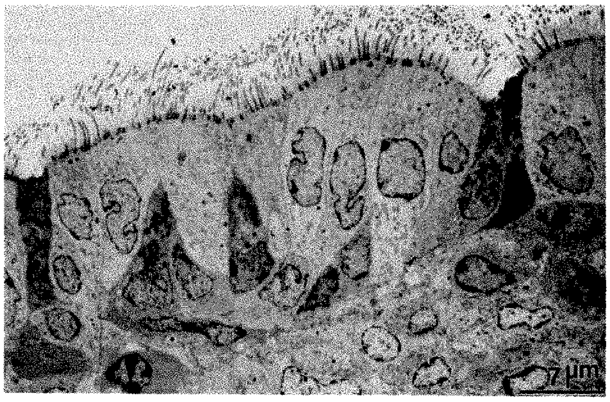

Fig 1. Transmission electron photomicrograph showing saline-inoculated proximal mucosa at day 3 (original x1,000). Ciliated cells, goblet cells, and basal cells are seen in epithelium. No pathologic ciliated cells are observed.

weight, 250 g), free of middle ear diseases as demonstrated by otoscopic examinations, were used. Under Nembutal anesthesia, a 10-μg/mL solution of LPS derived from *K pneumoniae* (Sigma) was inoculated into the right ear of each animal through the tympanic membrane until the solution overflowed from the tympanic membrane. By use of the same procedure, sterile physiologic saline solution was injected into the left ear of each animal to serve as a control. The animals were divided into three groups, each group containing ten animals. The three groups of animals were decapitated painlessly on day 1, 3, or 7 after the procedure. Bilateral mucosal samples were obtained from two sites within the tympanic cavity close to the tympanic orifice (site A, proximal site) and more distal to the orifice (site B, distal site) with least delay after decapitation. The details of their locations were described previously elsewhere.[11] The samples from five animals of each group were used for the assessment of ciliary activity (beats/minute), and those from the remaining five were prepared for electron microscopy.

Samples for examination of ciliary activity were kept in a medium of RPMI 1640 solution[16,17] until they were used for the examination. The ciliary activity in the most active cell of each sample was examined in a physiologic saline solution at an ambient temperature of 30°C, by use of the direct and quantitative photoelectric method of Ohashi and Nakai.[18,19] The ciliary activity in every sample was determined five times and the average value was used. About 20 minutes was required to examine the ciliary activity in each animal after decapitation. The data obtained were analyzed by Student's *t* test.

Samples for electron microscopy immediately were immersed in 2.5% cold glutaraldehyde in 0.1 *M* phosphate buffer solution for washing and were postfixed by 2% osmium tetroxide in the same buffer. After dehydration in ethanol, some of them were critical point dried, coated with gold, and observed under an S-405 scanning electron microscope. The remaining samples were embedded in epoxy resin after fixation and dehydration. Ultrathin sections were made with an ultramicrotome and examined under an H-300 transmission electron microscope.

RESULTS

SALINE-INOCULATED EARS (LEFT EARS)

On gross examination, no effusions were observed in each left ear of 30 animals at decapitation. The ciliary activities (mean ± standard deviations [SD]) in the proximal mucosa were 732 ± 35 beats/min at day 1, 753 ± 56 beats/min at day 3, and 743 ± 48 beats/min at day 7, respectively. Electron microscopy showed few pathologic findings in the proximal mucosa at day 1, day 3 (Fig 1), and day 7. The rate of pathologic ciliated cells showing compound cilia, vacuolation, or cytoplasmic protuberance was less than 5%.

The ciliary activities (mean ± SD) in the distal mucosa were 837 ± 42 beats/min at day 1, 853 ± 54 beats/min at day 3, and 822 ± 57 beats/min at day 7, respectively. Few pathologic findings were observed in the distal mucosa at any time examined. The ratio of pathologic ciliated cells revealing compound cilia, vacuolation, or cytoplasmic protuberance was less than 5%.

LIPOPOLYSACCHARIDE-INOCULATED EARS (RIGHT EARS)

Day 1. Massive serous effusions were found to fill the tympanic cavities of all animals completely. The ciliary activities were 420 ± 26 beats/min in the proximal mucosa and 466 ± 70 beats/min in the distal mucosa. Statistical analysis showed that the ciliary activities in the proximal mucosa and the distal mucosa of LPS-inoculated ears had deteriorated compared to those in the corresponding mucosa of the saline-inoculated ears at day 1 (proximal mucosa, $p < .0005$; distal mucosa, $p < .005$).

A number of pathologic findings were observed in the proximal mucosa under electron microscopy. The major pathologic signs included compound cilia, vacuolation of ciliated cells, infiltration of inflammatory cells (mainly polymorphonuclear neutrophil leukocytes [PMNs]), leakage of red blood cells from capillaries, and thickening of the subepithelial layer (Fig 2). The incidence of such degenerated ciliated cells was over 65%. Various pathologic findings also were observed in the distal mucosa. The conspicuous findings were compound cilia, infiltration of PMNs, vacuolation of ciliated cells, cytoplasmic protuberances, thickening of the subepithelial layer, leakage of erythrocytes, and sporadic sloughing of epithelial cells (Fig 3). The incidence of pathologic ciliated cells was over 65% also.

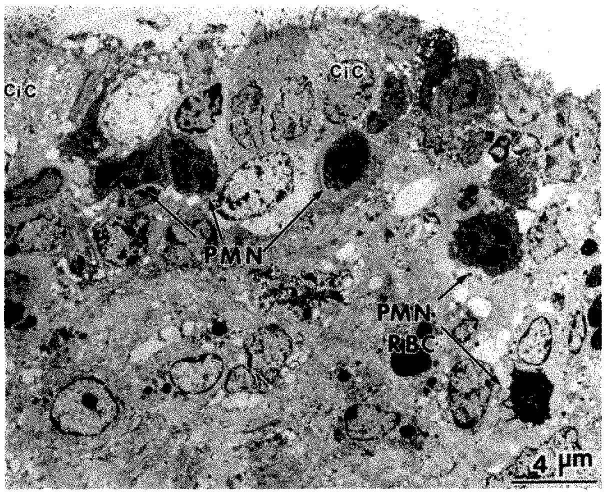

Fig 2. Transmission electron photomicrograph showing LPS-inoculated proximal mucosa at day 1 (original x1,500). Various pathologic findings including loss of cilia, invasion of polymorphonuclear neutrophil leukocytes (PMNs), vacuolation of ciliated cells (CiC), and leakage of red blood cells (RBC) are observed.

Day 3. Gross examination showed moderate effusions (average volume, 0.17 mL) in the tympanic cavities of all animals. The ciliary activities were 561 ± 76 beats/min in the proximal mucosa. The ciliary activities in the proximal mucosa were diminished compared to those of the saline-inoculated ears at day 3 ($p < .05$), but greater than those of the LPS-inoculated ears at day 1 ($p < .10$). The ciliary activities in the distal mucosa were 484 ± 64 beats/min, which were also statistically smaller than those of the saline-inoculated ears at day 3 ($p < .005$) and were not significantly different from those of the LPS-inoculated ears at day 1.

Several pathologic changes including compound cilia, vacuolation of ciliated cells, cytoplasmic protuberances, and infiltration of PMNs were observed in the proximal mucosa (Fig 4A). The incidence of pathologic ciliated cells was approximately 45%. Electron microscopy showed various pathologic findings in the distal mucosa similar to those found in the LPS-inoculated ears at day 1. These findings included infiltration of PMNs, compound cilia, leakage of erythrocytes, and vacuolation of epithelial cells (Fig 4B). The incidence of pathologic ciliated cells was approximately 55%.

Day 7. At day 7, fluid could hardly be detected in the tympanic cavity of each animal. The ciliary activities in the proximal mucosa were 699 ± 62 beats/min, which were not different from those of the saline-inoculated ears. On the other hand, the ciliary activities in the distal mucosa were 527 ± 55 beats/min. Statistical analysis showed that the ciliary activities in the distal mucosa were significantly smaller than those of the saline-inoculated ears at day 7 ($p < .005$) and were not different from those of the LPS-inoculated ears at day 1 or day 3.

Electron microscopic examinations showed sporadic pathologic signs such as vacuolation of ciliated cells in the proximal mucosa (Fig 5A). However, the incidence of such degenerated ciliated cells was less than 10%. On the other hand, electron microscopy showed some pathologic findings in the distal mucosa that included sporadic infiltration of inflammatory cells, compound cilia, vacuolation of ciliated cells, and thickening of the subepithelial layer (Fig 5B). The incidence of such degenerated ciliated cells was approximately 40%.

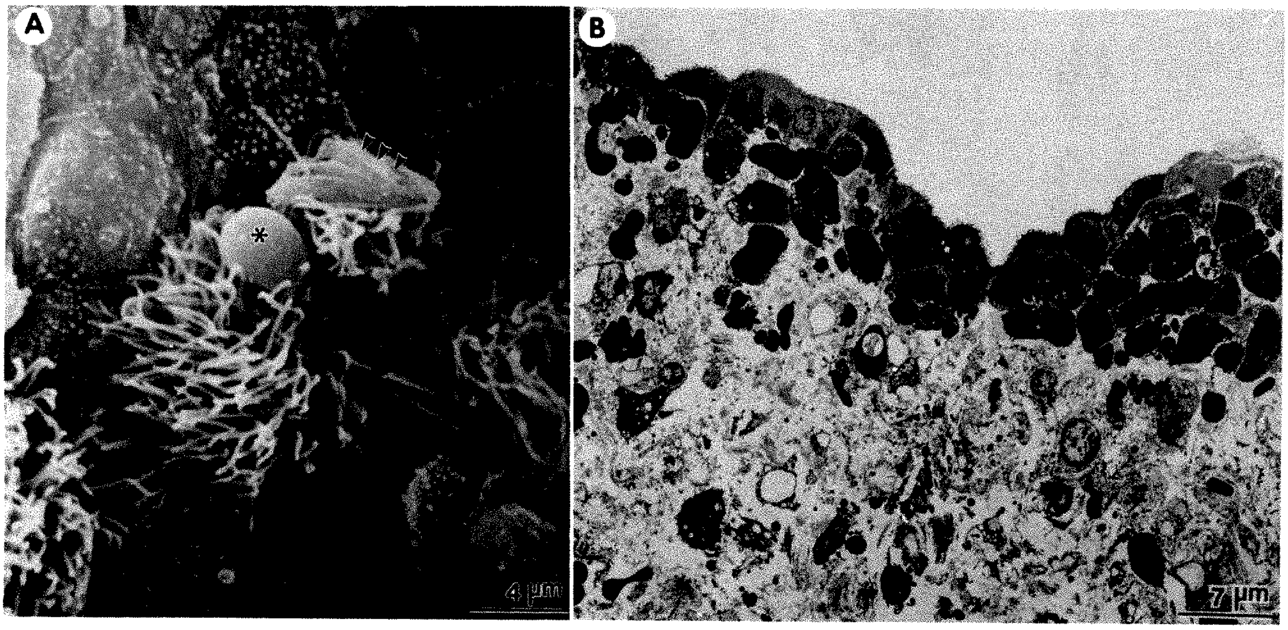

Fig 3. Pathologic findings in LPS-inoculated distal mucosa at day 1. A) Scanning electron photomicrograph (original x3,500). Compound cilia (arrowheads) of adhesive type and cytoplasmic protuberances of epithelial cells (asterisk) are seen. B) Transmission electron photomicrograph (original x700). Erythrocytes that have escaped from blood vessels and infiltration of numerous PMNs are observed.

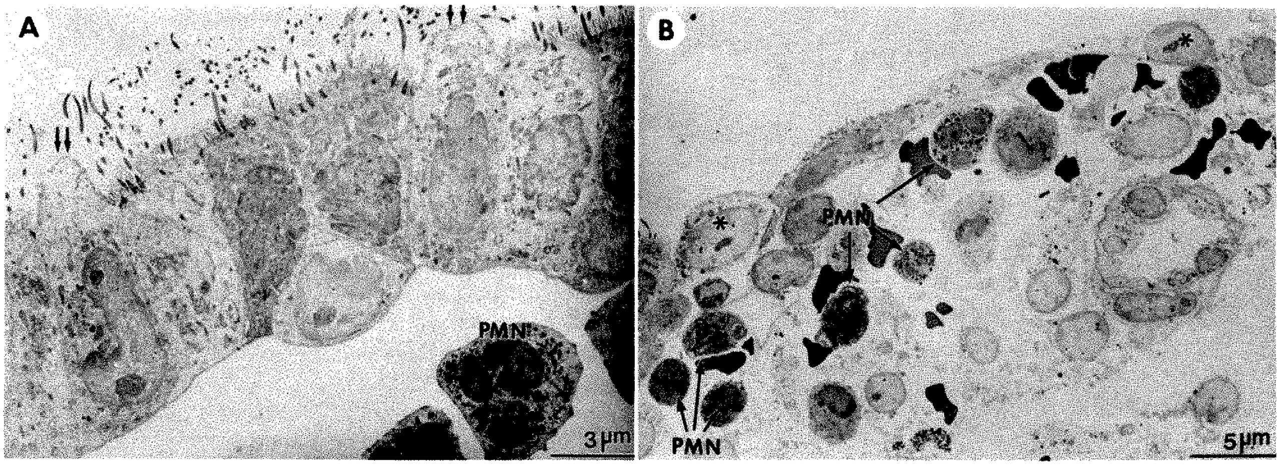

Fig 4. Transmission electron photomicrographs showing LPS-inoculated mucosa at day 3. A) Proximal site (original x2,000). Sporadic cytoplasmic protuberances of nonciliated cells (arrows) and PMNs in subepithelial layer are seen. B) Distal site (original x2,500). In subepithelial layer, numerous erythrocytes that have escaped from vessels and many PMNs are observed. Some nonciliated cells (asterisks) show cytoplasmic vacuolation.

DISCUSSION

Otitis media with effusion, or secretory otitis media, is one of the most prevalent pathologic conditions encountered in pediatric otologic practice. This disease is characterized by the accumulation of fluid in the middle ear, which frequently can cause deafness at a critical time in a child's development and can interfere with speech development and social interaction. In an attempt to elucidate the mechanisms or pathogenesis of this disease, several investigators tried to induce this condition in laboratory animals by various procedures. Of them, two ways that were successful in evoking effusion in the tympanic cavity were mechanical obstruction of the eustachian tube and direct inoculation of bacteria or bacterial elements. These procedures resulted in MEEs in a large number of cases, and under these experimental conditions, the pathology of OME has been well studied by morphologic techniques. It has been confirmed that increased vascular permeability and vascular injury are the responsible mechanisms for MEE production.[4,5] However, any effusion formed in the tympanic cavity should be removed to the pharynx if the mucociliary system in the tubotympanum is functioning normally. Indirect evidence has been accumulating to the effect that a damaged or inflamed mucociliary apparatus in OME might be associated with insufficient clearance in the middle ear.[5,20]

However, our knowledge of the mucociliary system in the tubotympanum is still limited. It has been only recently that the mucociliary system has been considered to play an important role in the defense system of the tubotympanum. Sadé[21] was the first to demonstrate, by tracing the movement of foreign materials (visible charcoal particles or glass

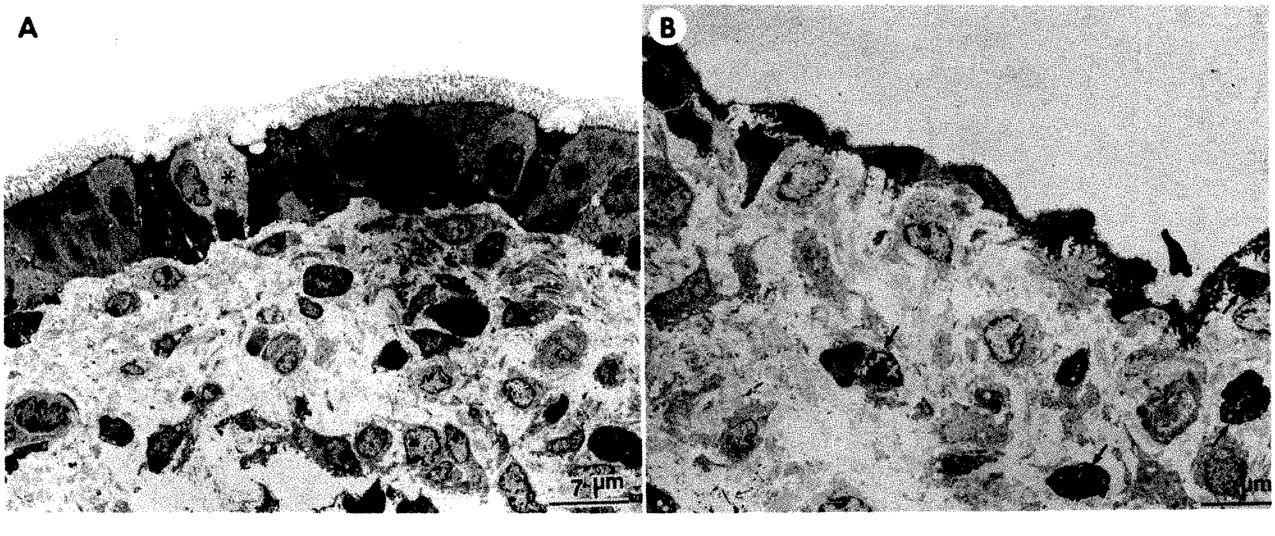

Fig 5. Transmission electron photomicrographs showing LPS-inoculated mucosa at day 7. A) Proximal site (original x700). Vacuolated ciliated cells (asterisk) are infrequently seen. Most epithelial cells show normal morphology. B) Distal site (original x1,500). Sporadic PMNs (arrows) and thickening of subepithelial layer are recognized, but nonciliated cells are normal.

beads), that the middle ear has an active transport function. Hadas and Sadé[22] measured the mucociliary clearance rate in patients with middle ear diseases by the use of nonsoluble saccharin and demonstrated that most ears with "simple" chronic otitis media and atelectatic ears have a patent eustachian tube and their mucociliary system can transport foreign particles through it. They also demonstrated that the transport tends to be slowed down if the ear is infected. Recently, Karja et al[23] and Nuutinen et al[24] measured the mucociliary clearance in OME with a radioisotopic method. They established that the mucociliary transport was declined or totally absent in OME and that the mucociliary transport returned to normal when the ear was clinically healed. These studies showed that the impairment of the mucociliary system in the tubotympanum plays an important role in the development and pathogenesis of OME.

Since the mucociliary system is composed of ciliated cells as well as secretory cells and a mucus blanket, impaired mucociliary function is due not only to mucus abnormalities but also to lowered ciliary activity. The effect of pathologic mucus on the mucociliary function in the middle ear has been well elucidated by a recent sophisticated study.[25] It is necessary to study the ciliary activity, a component function of mucociliary clearance, to gain a better understanding of the nature of this system. However, the ciliary activity in the middle ear has received little consideration. Our study has documented this activity directly and quantitatively by use of our photoelectric technique. We demonstrated that ciliary activity exists in the middle ear, similar to its presence in respiratory organs, and the ciliary activity is stronger (ie, more beats per minute) as the tympanic location becomes more distal to the eustachian tube.[9,10] Our investigation has provided new and direct means to evaluate the biologic defense system in the middle ear. Using the same technique, we have studied the effects of a variety of pathologic agents on the ciliary activity in the tubotympanum. These included x-ray irradiation,[12,26-28] infectious pathogens,[11,13-15,29] and environmental pollutants.[30-32] In these studies, we have established that the ciliary activity in the tubotympanum can be reduced by a variety of pathologic agents. We have studied the effect of LPS derived from *K pneumoniae* on the ciliary activity in the in vitro middle ear mucosa and we have confirmed that LPS can affect the ciliary activity in a dose-response fashion[14]: 1) LPS does not affect the ciliary activity up to 168 hours if the concentration is 1 ng/mL or less; 2) 10 ng/mL of LPS does not impair ciliary activity in the middle ear close to the tympanic orifice up to 168 hours, but can cause reduced activity in the middle ear distal to the orifice after extended exposure (more than 96 hours); 3) 100 ng/mL or more of LPS can cause dysfunction of cilia in the tympanic cavity, and in particular 1 μg/mL or more of LPS can quickly disable the ciliary activity in the middle ear distal to the orifice. However, the possibility cannot be ruled out that the ciliated cells in situ could behave differently when in contact with LPS. Thus, we inoculated the tympanic cavity of guinea pigs with LPS from *K pneumoniae* in the present study and examined the beating activity as well as the structure of ciliated cells under such conditions.

In this study we found serous effusions exclusively in the tympanic cavity of the LPS-inoculated ears at days 1 and 3. At day 1 and day 3, we found reduced ciliary activity in the LPS-inoculated ears. We immersed mucosal samples in RPMI 1640 solution immediately after sampling. It has previously been established that the ciliary activity in the middle ear shows no significant decrease in the presence of RPMI 1640 solution for periods of more than 24 hours.[14,15,17] Thus, the reduction of ciliary activity in the LPS-inoculated ears is not due to postmortem changes but to the effect of LPS inoculation. Our speculation also is supported by a comparison with the ciliary activity in the control (saline-inoculated) ears.

In addition to such functional examinations, an increased number of pathologic ciliated cells in the LPS-inoculated ears was confirmed by morphologic examinations using scanning and transmission electron microscopy. It seems apparent that diminished ciliary activity in the middle ear is in all probability responsible for the accumulation of fluid in the tympanic cavity. At day 7, however, fluid could hardly be detected even in the LPS-inoculated ears, when the ciliary activity in the distal mucosa was reduced but the ciliary activity in the proximal mucosa had returned to normal. It has been established that serous MEE formation due to endotoxin or LPS is the result of increased vascular permeability.[4,5] In consideration of these studies and our present data, it may be presumed, therefore, that two specific pathologic factors are needed in the development and manifestation of OME — MEE production due to increased vascular permeability and failure of MEE transport due to impaired mucociliary activity. It also may be assumed that the cilia in the middle ear close to the orifice play an even more significant role for middle ear clearance.

In this study, significant discrepancy in the susceptibility of the ciliary activities to LPS was observed between two locations. In this connection, we have already reported a similar discrepancy in reactions to radiography,[12,26-28] infectious pathogens,[11,13-15,29] and toxic gases.[30-32] The effects of most pathologic factors were larger on the ciliary activity in the distal mucosa than on that in the proximal mucosa. It seems logical to assume that the cilia in the distal mucosa are much more susceptible to various pathologic factors than those in the proximal mucosa. The present histologic examinations using electron microscopy also have disclosed that the cilia or ciliated cells in the distal mucosa are damaged more dramatically than those in the proximal mucosa; this finding corresponds well to the functional data.

In our previous studies using in vitro laboratory models, 10 μg/mL of LPS showed much larger cilioinhibitory or ciliostatic effects.[13-15] Discrepancies between the effects of LPS on the in vitro cilia and on the in vivo cilia may be assigned to the buffering function of the mucus blanket. The findings of this study combined with those of previous studies made in our laboratory suggest that the mucus blanket covering the cilia may lessen the ciliotoxic effects of pathologic agents including LPS.

In conclusion, our present investigation shows that LPS or endotoxin extracted not only from *H influenzae* but also from other kinds of bacteria may be involved in the clinical development of OME and that the dysfunction of cilia caused by endotoxin is a responsible factor for the condition. It is also worthy of mention that the mucociliary system is indeed an important defense system; that the failure of such a system, especially in the middle ear close to the tympanic orifice, could lead to a retention of effusions in the ear; and that improvement of such a system may result in clinical benefit.

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IMAGING CASE STUDY OF THE MONTH

CARCINOMA OF THE MAXILLARY SINUS WITH PTERYGOID INVASION

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Carcinoma of the maxillary sinuses is associated with a poor prognosis because of diagnosis in advanced stages of the disease, high recurrence rates, and close proximity to vital structures. These tumors have been staged during operative exploration via a Caldwell-Luc approach to the sinuses. Recently, computed tomography has been used for staging according to the American Joint Committee for Cancer Staging.[1-3] Kondo et al[2] showed that CT scan was as good as maxillectomy in staging tumors, but that exploratory maxillotomy (Caldwell-Luc) was suboptimal for accurate staging of tumors demonstrating deep invasion.[2,4] Staging by CT can be difficult in certain cases in which actual soft tissue invasion cannot be demonstrated. Accurate tumor staging requires determination of invasion of soft tissue structures such as the pterygoid muscle, skin of the cheek, and orbit.

We report a case of carcinoma of the maxillary sinus that invaded the infratemporal fossa. Magnetic resonance imaging demonstrated pterygoid muscle invasion and aided in staging, preoperative surgical planning, and informed consent.

CASE REPORT

A 75-year-old man presented to another institution with a 13-month history of nasal obstruction and a left nasal mass. Transnasal biopsy and CT scan revealed a T2N0M0 squamous cell carcinoma of the left maxillary sinus. The patient underwent radiotherapy, receiving 6,400 rad. Eight months later, radiographs demonstrated erosion of the posterior wall of the sinus, and repeat biopsy revealed squamous cell carcinoma. The patient complained of left facial pain and trismus that was increasing in severity. The patient was referred to our service for further management. Both CT and MRI were performed. The CT revealed extension of the tumor medially into the nasal cavity and posteriorly into the infratemporal fossa (see Figure, A). The MRI (T2-weighted image) revealed tumor invasion of the lateral pterygoid muscles (see Figure, B). There was increased contrast between muscle and tumor, with the hyperintense signal of the tumor being noted on the T2-weighted image. With this information, preoperative surgical planning included plans for an infratemporal fossa dissection in addition to maxillectomy. At surgery, the tumor was noted to have destroyed the posterior wall of the maxillary sinus and invaded the lateral pterygoid muscle. A Weber-Ferguson incision was used to gain exposure. Radical maxillectomy; infratemporal fossa dissection; and excision of the tumor, pterygoid muscle, and pterygoid plate were performed. Postoperatively the patient had an uneventful course.

DISCUSSION

Carcinoma of the maxillary sinus frequently invades the bony walls of the sinus, impinging upon adjacent vital structures (orbit, pterygoid muscle, etc). A CT scan can demonstrate bony involvement and tumor expansion, but actual soft tissue invasion may be difficult to interpret. An MRI scan can provide superior soft tissue contrast and vascular imaging without radiation exposure or need for contrast infusion. When muscle is invaded by tumor, there is a change in signal intensity that further enhances the contrast between muscle and tumor.[5] This phenomenon was most prevalent on the T2-weighted images as seen with our patient. An MRI is sensitive to orbital invasion because of its ability to demonstrate disruption of normal fatty planes and normal soft tissue relationships.

In this case, MRI findings of lateral pterygoid muscle invasion accounted for the patient's complaints of trismus. On CT scan, the tumor was seen invading the infratemporal fossa, but the presence of pterygoid muscle invasion was difficult to assess. On MRI, the tumor was hyperintense compared to the pterygoid muscle, which was isointense. The actual muscle invasion was well demonstrated (see Figure, B).

Preoperative knowledge of infratemporal fossa extension with pterygoid muscle invasion permitted preparation for the combined surgical approach to the maxilla and infratemporal fossa. The patient

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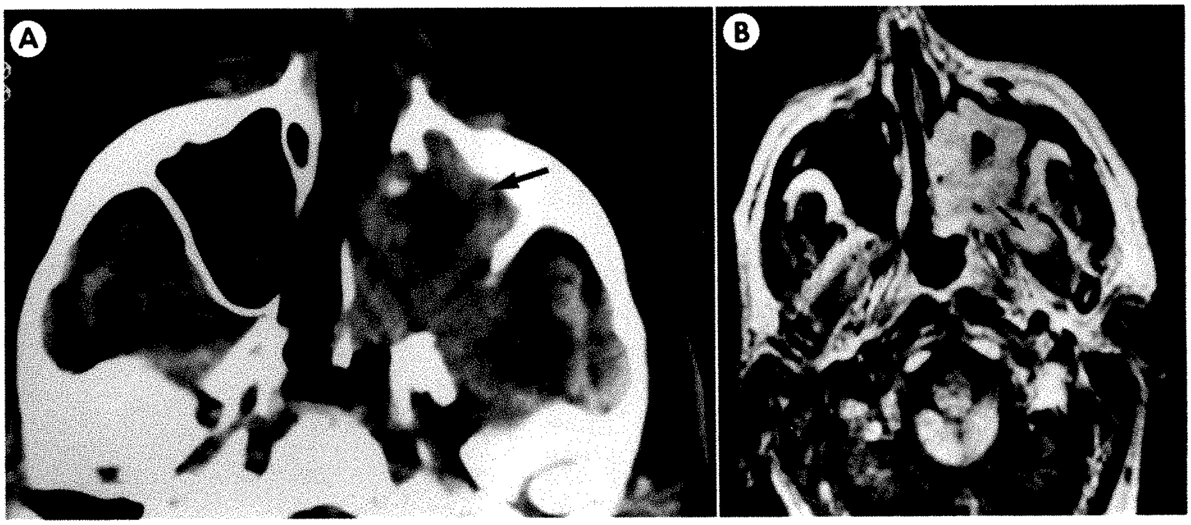

Carcinoma of maxillary sinus. A) Axial post-contrast computed tomograph showing isodense mass obliterating left maxillary sinus, with bony destruction and medial extension into nasal cavity and posterior extension into infratemporal fossa. Arrow — tumor. B) Axial T2-weighted magnetic resonance image demonstrating hyperintense tumor of left maxillary sinus. Tumor extends into infratemporal fossa. Note hyperintense tumor (arrow) invading isointense muscle.

was informed of the extent of the tumor and the magnitude of surgery that would be necessary to completely excise the tumor. A Weber-Ferguson incision provided good exposure of the nasal cavity, maxilla, floor of the orbit, and ipsilateral zygoma. At the time of surgery, the left zygoma was removed to permit exposure of the infratemporal fossa after radical maxillectomy was performed. A temporalis muscle flap was sutured medially to the nasal bone to act as a sling to support the orbit from below.

The use of CT and MRI to study maxillary sinus cancer can provide a tremendous amount of preoperative data pertaining to 1) location of the bulk of tumor, 2) degree of bony invasion, 3) invasion of vital structures, and 4) presence or absence of metastatic disease. The studies complement each other and can aid in staging maxillary sinus carcinoma. Accurate staging will help prevent the misuse of multimodality therapy, which frequently differs according to tumor stage.

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PATHOLOGY CONSULTATION

SIALOCYSTS OF THE PAROTID GLANDS

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Sialocysts of salivary glands are outnumbered greatly by pseudocystic lesions, exemplified by mucoceles. In the major salivary glands, sialocysts are preponderantly lesions of the parotid glands, where they are classified as 1) salivary duct cysts, 2) lymphoepithelial cysts, or 3) dysgenetic or congenital cysts. All arise from salivary ducts and have no relation with the branchial apparatus.

Epithelial-lined, nonneoplastic cysts of salivary glands are relatively rare when compared with the common mucus retention phenomenon or mucocele. The latter is a pseudocyst without an epithelial lining, has a traumatic or obstructive basis, and is preponderantly a lesion of minor salivary glands of the lips and oral cavity.

Sialocysts are true (epithelial-lined) cysts of salivary duct origin and may be acquired, secondary to stasis of secretion, or congenital. In the oral cavity, the buccal mucosa and floor of mouth are the sites of predilection. In the major salivary glands, sialocysts are primarily lesions of the parotid gland. The true incidence of these parotid cysts is impossible to relate, but the results of Seifert et al[1] and Seifert and Waller[2] give a general indication (see Table[1]).

The benign sialocysts of the parotid gland fall into three basic histologic-histogenetic groups: 1) salivary duct cysts, 2) lymphoepithelial cysts, and 3) dysgenetic or congenital cysts. Salivary duct cysts are the most common and are no more than unilocular or occasionally multilocular ectatic salivary ducts with or without an oncocytic epithelial lining. They occur randomly in the parenchyma from intralobular or interlobular ducts and are unassociated with a lymphoid component. Lymphoepithelial cysts always have an associated lymphoid tissue, have a variable epithelial lining, and in the oral cavity most often are found in the floor of the mouth.[3]

Two principal and conflicting mechanisms of development of lymphoepithelial cysts have been proposed: branchiogenic origin and cystic dilation of enclaved salivary inclusions within lymph nodes or intrinsic lymphoid tissue.[4,5] "Branchial" has been indiscriminately applied to a variety of cysts in and about the parotid glands. The intimate association of the cysts with lymphoid tissue or an intranodal location, and their squamous linings, have led to the assumption of a branchiogenic origin. This is very unlikely. The branchial apparatus has almost completely disappeared by the 45th day of fetal life — when parotid gland development usually is beginning.[5] The first branchial cleft defect, in effect a duplication anomaly of the external auditory canal, may be associated with the preauricular part of the parotid gland and the facial nerve, but is histologically unlike the lymphoepithelial cyst.[6,7] The first cleft cyst/sinus has no lymphoid component, is lined with a keratinizing squamous epithelium (epidermis), and often has cutaneous adnexal structures and cartilage.

The embryologic origin and migration of the parotid anlagen, the nearly constant finding of enclaved salivary ducts and acini in intraparotid and periparotid lymph nodes, a high amylase content in the cyst fluid, and salivary sialoliths in some cysts all speak to salivary inclusions as precursors to lymphoepithelial cysts.[2,8] With the exception of an oncocytic cell component, the histogenesis and appearance of the lymphoepithelial cyst are those of a Warthin's tumor. Typically, the epithelial-lined cysts are surrounded by abundant lymphoid tissue or are within a lymph node (Fig 1). The lining ranges from cuboidal duct cells to a metaplastic squamous epithelium.[9] Keratinization is not common. Mucous cells may be interspersed. The lym-

NONNEOPLASTIC CYSTIC LESIONS OF SALIVARY GLANDS

Salivary Glands Most Involved	*Type of Cyst*	*Percent of Total*
Minor salivary glands of oral cavity and lip	Mucocele	76
Parotid gland	Salivary duct	9
Parotid gland and oral cavity	Lymphoepithelial	7
Sublingual gland	Ranula	6
Parotid	Congenital sialectasis	1.5
Parotid	Polycystic (dysgenetic)	0.5

From a study of 360 cystic lesions by Seifert et al.[1]

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REPRINTS — John G. Batsakis, MD, Division of Pathology, University of Texas M. D. Anderson Cancer Center, 1515 Holcombe Blvd, Houston, TX 77030.

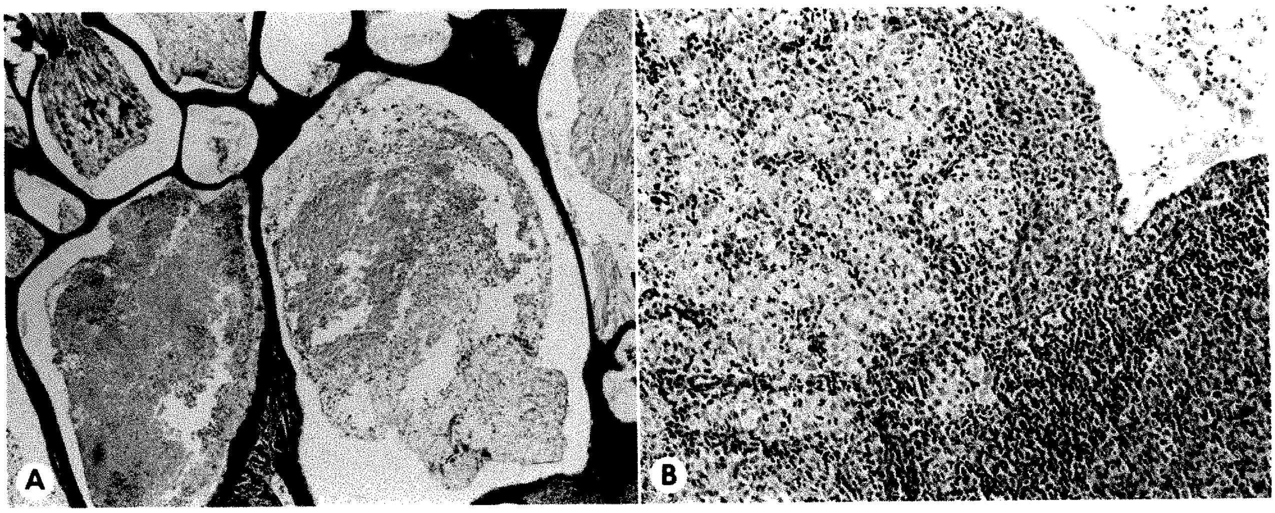

Fig 1. Lymphoepithelial cysts. A) Multiloculated cyst within parotid lymph node (H & E, original x30). Thin lymphoid septae separate dilated cystic spaces lined with flattened epithelium and containing keratin-granular and secretory debris. B) Unilocular cyst of parotid gland (H & E, original x140). Cyst is intranodal, manifests metaplastic epithelium, and has reactive follicle center and clusters of epithelioid cells in lymphoid stroma.

phoid tissue is indigenous and not inflammatory, but may show focal granulomas and reactive follicles.

The neoplastic and malignant potential of parotid cysts is minimal, but the lymphoepithelial cyst may be a progenitor for some of the rare cystic primary squamous cell carcinomas of the parotid gland.

Congenital cystic disease of the parotid glands is rare. It takes two imperfectly separated forms: dysgenetic polycystic disease and congenital sialectasis. An indication of the rarity of polycystic parotid disease is evinced by the finding of only two examples from a total of 5,739 cases of disorders of salivary glands studied by Seifert et al.[1] The dysgenetic cystic parotid gland is a polycystic malformation of the salivary duct system. The disorder may be unilateral or bilateral. It is believed that intrauterine disturbances in the ramification and canalization of the terminal salivary buds are responsible.[1] In that regard, the abnormality is comparable to the developmental changes leading to cystic malformations of the pancreas or lungs. Whether patients with polycystic parotid disease also have cystic anomalies in other parenchymal organs is unknown. Clinical manifestations may be long delayed. One of the two patients presented by Seifert et al[1] was 65 years old, and Fig 2 shows a lesion involving only the deep lobe of the parotid gland in a 32-year-old woman. The polycystic parotid gland has a honeycombed appearance on low magnification. There is preservation of the lobular architecture, but acinar parenchyma remains only as is-

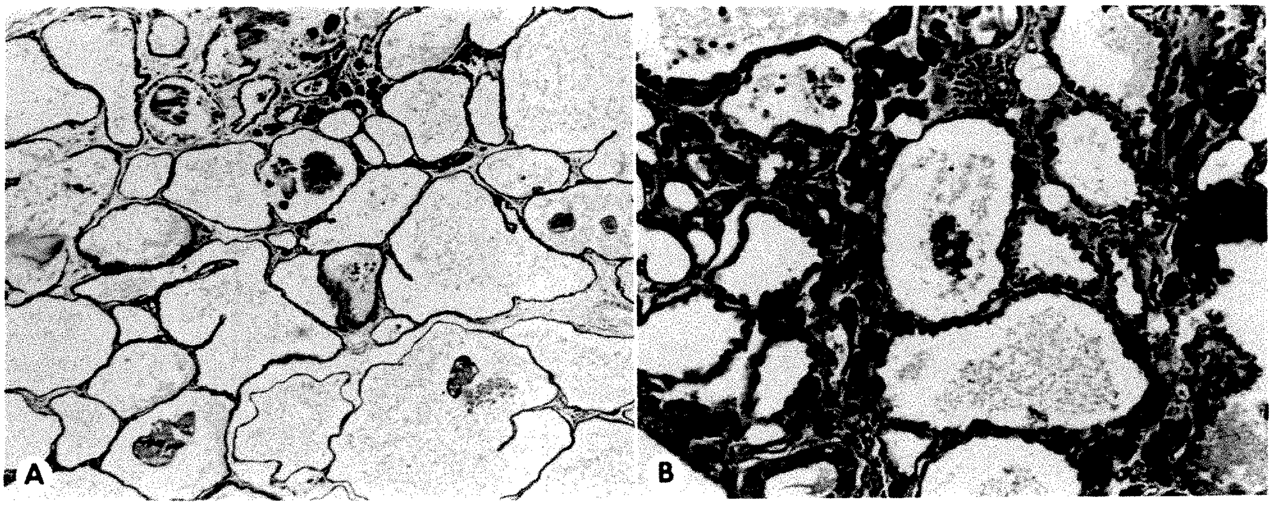

Fig 2. Polycystic disease of parotid gland. A) Lymphaticlike spaces have replaced and compressed acinar salivary parenchyma (H & E, original x30). Cystic spaces contain inspissated secretions and sialoliths. B) Higher magnification (H & E, original x140). This field shows cuboidal lining cells of cystic spaces separated by thin acinar parenchyma.

lands in the cystically transformed lobules. The lining of the cysts varies from flattened endothelium-like cells to cuboidal or larger cells with secretory granules. An inflammatory reaction is absent, but many of the cystic ducts contain inspissated salivary secretions, spheroliths, and microliths.

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SCLEROSING ORBITAL PSEUDOTUMOR

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SELECTIVE REINNERVATION OF BDUCTOR AND ADDUCTOR MUSCLES OF CANINE LARYNX AFTER RECURRENT NERVE PARALYSIS

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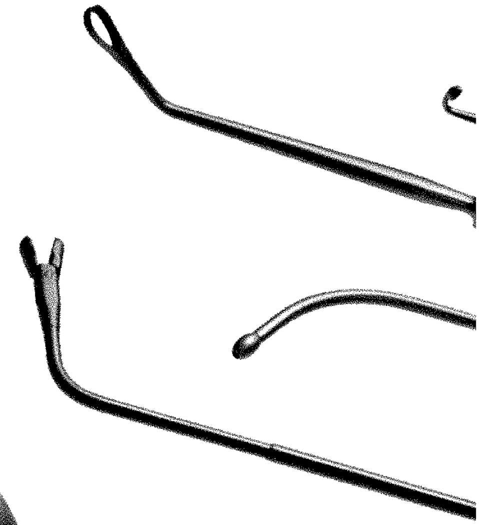

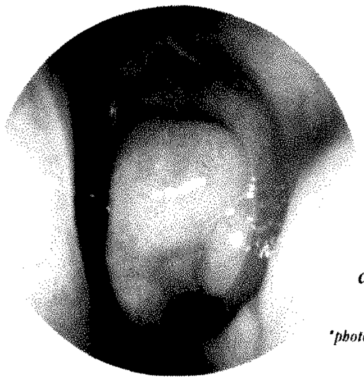

*Concha bullosa on left side bulging into lateral nasal wall. Entrance to middle meatus blocked and a polyp is arising from the contact area.**

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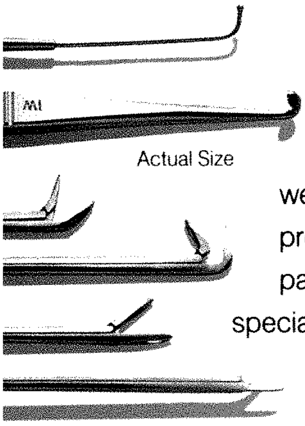

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ROLE OF PERILYMPHATIC FISTULA IN SUDDEN HEARING LOSS: AN ANIMAL MODEL

ERIC M. OSHIRO

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STANFORD, CALIFORNIA

The electrophysiologic response of the guinea pig cochlea was monitored after sequential lesions to Reissner's membrane and the round window (RW). Action potential (AP) responses to click stimuli were recorded from the RW before and after discrete puncture-type lesions were created in the cochlear partition of the second turn. Observed decrements were typically minor, comparable to no greater than 10 dB attenuation of stimulus intensity. The RW membranes then were perforated to create perilymphatic fistulas. Further monitoring demonstrated a rapid (within 5 to 10 minutes), severe decrement in AP amplitude and latency, with complete loss of the AP within 1 hour. Control animals with RW perforations alone did not show these decrements. Correct placement of the second turn lesions was documented by histology. We conclude that discrete lesions in the cochlear duct are not reflected in the AP input-output functions unless there is a fluid leak from the RW, and thus present a possible model for idiopathic sudden hearing loss.

KEY WORDS — cochlea, endolymph, perilymphatic fistula.

INTRODUCTION

The idea that perilymphatic fistulas (PLFs) or intracochlear membrane breaks might play a role in the genesis of idiopathic sudden hearing loss (SHL) has been a central concept in theories of SHL.[1,2] However, the patterns of hearing loss, and sometimes recovery, in SHL continue to remain an enigma as a clinical entity, while several lines of evidence seem to indicate that a perilymph leak alone cannot explain the sometimes severe deficits seen in SHL.

Clinically, for example, PLFs commonly are seen in the absence of hearing loss (F. B. Simmons, personal communication, 1987). Animals studies have shown that cochlear tuning curves and action potential (AP) N1 essentially remain unaltered after opening of the round window (RW).[3,4] Finally, three-dimensional mathematical models demonstrate that basilar membrane mechanics are not significantly affected by opening and draining of the cochlea through the RW.[5]

It has been proposed that the deficits seen in SHL can be explained by damage, or another break, elsewhere in the cochlear duct—for example, a Reissner's membrane lesion.[6] In this case, admixture of perilymph and endolymph might be expected to cause only localized functional disruption in the cochlea, if fluid flow patterns were predominantly radial. Widespread admixture of fluids with larger functional deficits, implying longitudinal flow, might be created by the PLF itself. This argument, therefore, rests on the premise that endolymph flow is primarily radial in the normal situation and that the drop in perilymph pressure caused by the PLF is sufficient to induce longitudinal flow.

Although investigators classically have disagreed as to whether the pattern of endolymph flow is primarily radial or longitudinal, the bulk of the evidence indeed seems to support the radial flow theory, which holds that endolymph is both produced and resorbed locally in the scala media. Consistent with this theory, there is documentation that deficits in the cochlear microphonic (CM) and AP remain localized after discrete lesions of Reissner's membrane[7,8] and that histologic degeneration of both the organ of Corti and stria vascularis remains localized even several months following a discrete Reissner's membrane lesion.[9-11] Furthermore, tracer experiments using labelled ions have documented that Reissner's membrane is permeable to potassium and sodium, although there is no evidence for significant active ion transport across this barrier.[12] Electron microscopy studies have demonstrated anionic sites on Reissner's membrane that may play the role of a size- and charge-selective barrier between the scala media and the scala vestibuli.[13] Taken together, these studies are highly suggestive of a radial component in the dynamic equilibrium of electrolytes in the cochlear fluids.

The longitudinal flow theory proposes that endolymph is produced by the stria vascularis or contiguous structures and travels down the scala media, through the ductus reuniens to the saccule, and through the endolymphatic duct to the endolymphatic sac, where it is absorbed.[14] In support of this theory Guild[14] demonstrated that iron salts injected into the scala media could be precipitated in the endolymphatic sac when the animals were killed. Indirect evidence for longitudinal flow has been demonstrated by Kimura,[15] who has shown that surgical obliteration of the endolymphatic duct

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From the Division of Otolaryngology, Stanford University School of Medicine, Stanford, California. Supported by National Institutes of Health grant NS 10532.

and sac results in endolymphatic hydrops in chronic guinea pig preparations. More recently, Salt et al[16] demonstrated by a method of iontophoretic injection of tracer ions into the scala media a measurable but slow (< 1 nL/min) rate of longitudinal endolymph flow toward the basal turn after bolus injection of tracer. Moreover, they demonstrated by comparison with an analytic model that the observed spread of tracer ion within the scala media is dominated by passive diffusion processes with very little contribution from longitudinal endolymph flow.

If the previous data can be summarized at all, one must acknowledge evidence for both radial and longitudinal components of endolymph flow, realizing, of course, that the two patterns of flow are not mutually exclusive. The data do, however, seem to indicate that the radial pattern predominates, since experimental damage to the cochlear duct, whether mechanical or due to fluid admixture, remains localized in both acute and chronic preparations, and that longitudinal flow by all measures must be considered relatively slow. These conclusions are relevant to the study of SHLs in that hypotheses that invoke perilymph and endolymph admixture as a basis for physiologic deficits require, as corollary, some explanation for longitudinal fluid flow.

The experiments described here attempt to characterize endolymph flow in a special situation, namely, the situation described above in which a perilymph leak is created in the presence of a Reissner's membrane fenestra. The electrophysiologic response of the guinea pig cochlea was monitored as a method of following cochlear deficits after sequential lesions to Reissner's membrane and the RW membrane in an attempt to model the proposed theory of SHL.

MATERIALS AND METHODS

Pigmented guinea pigs weighing 500 to 800 g were anesthetized with ketamine hydrochloride (50 mg/kg) and acepromazine (20 mg/kg) administered intramuscularly. Animals were placed in a lateral position with the experimental ear facing upward. Lidocaine (1%) was infiltrated into the area of surgical incision. The bulla was opened and a 3-mil platinum wire, ball-tipped recording electrode was placed on the bony niche of the RW. The external auditory canal then was transected at the external meatus and the pinna was reflected forward.

With cochleas intact, the animals were placed in a recording booth and click stimuli 100 μs in duration were delivered at 20/s from a distance of 1 cm to the external auditory meatus free-field through an earphone (Sony MDR-A10). Action potential and CM response were amplified differentially (Grass P511K), digitally averaged, and recorded (DEC PDP-11). Responses were measured as a function of increasing stimulus level in 10-dB increments, with maximum output voltage calibrated at 104 dB peak sound pressure level with a probe microphone (Bruel & Kjaer 4133).

A discrete puncture-type lesion then was created in Reissner's membrane of the second cochlear turn by inserting a 1-mil stainless steel wire probe into a hole drilled into the scala media. The pigment of the stria vascularis was used as a landmark for placement of this lesion. The bony cochlea was sealed with bone wax and the AP measurements were repeated several times over the next 30 to 60 minutes while we looked for changes in the AP or CM correlating with the Reissner's membrane fenestra.

A PLF then was created by perforating the RW membrane with a stainless steel probe, with lesions verified by visual inspection. Action potential measurements were monitored up to 1 hour after the RW lesion while we looked for changes in both latency and amplitude of N1. Cochleas were fixed via intracardiac perfusion, and placement of the second turn lesions was verified by histologic preparations. Controls consisted of simple RW membrane puncture, after which the AP and CM response was monitored for 1 hour. Decrements in the potentials after RW puncture in both groups did result from perilymph pooling around the electrode in the RW niche; however, responses returned to baseline when this fluid was wicked away with cotton.

A total of 28 animals were used for the entire experiment. The first ten animals were used for the development of the surgical and recording technique. The results reported here represent seven ears from five experimental animals and six control ears from six control animals.

RESULTS

Following discrete lesions in Reissner's membrane, decrements in the AP N1 response or the CM, when they were observed, were typically minor, corresponding to changes comparable to 5- to 10-dB attenuation of the click stimuli. In two of the seven animals there was actually an increase in the amplitude of the AP after the lesion. Input-output functions constructed with the averaged data demonstrate that after the Reissner's membrane lesion changes in N1 amplitude, CM amplitude, or shift in N1 latency over the 30- to 60-minute period remained minimal (Fig 1). The post–Reissner's membrane lesion data in Fig 1 represent averaged data from the last measurement from individual animals during this 30- to 60-minute period.

When this discrete Reissner's membrane lesion was followed by a RW puncture, there was a severe drop in the amplitude of N1 and CM and a marked shift in the N1 peak latency that developed within 5

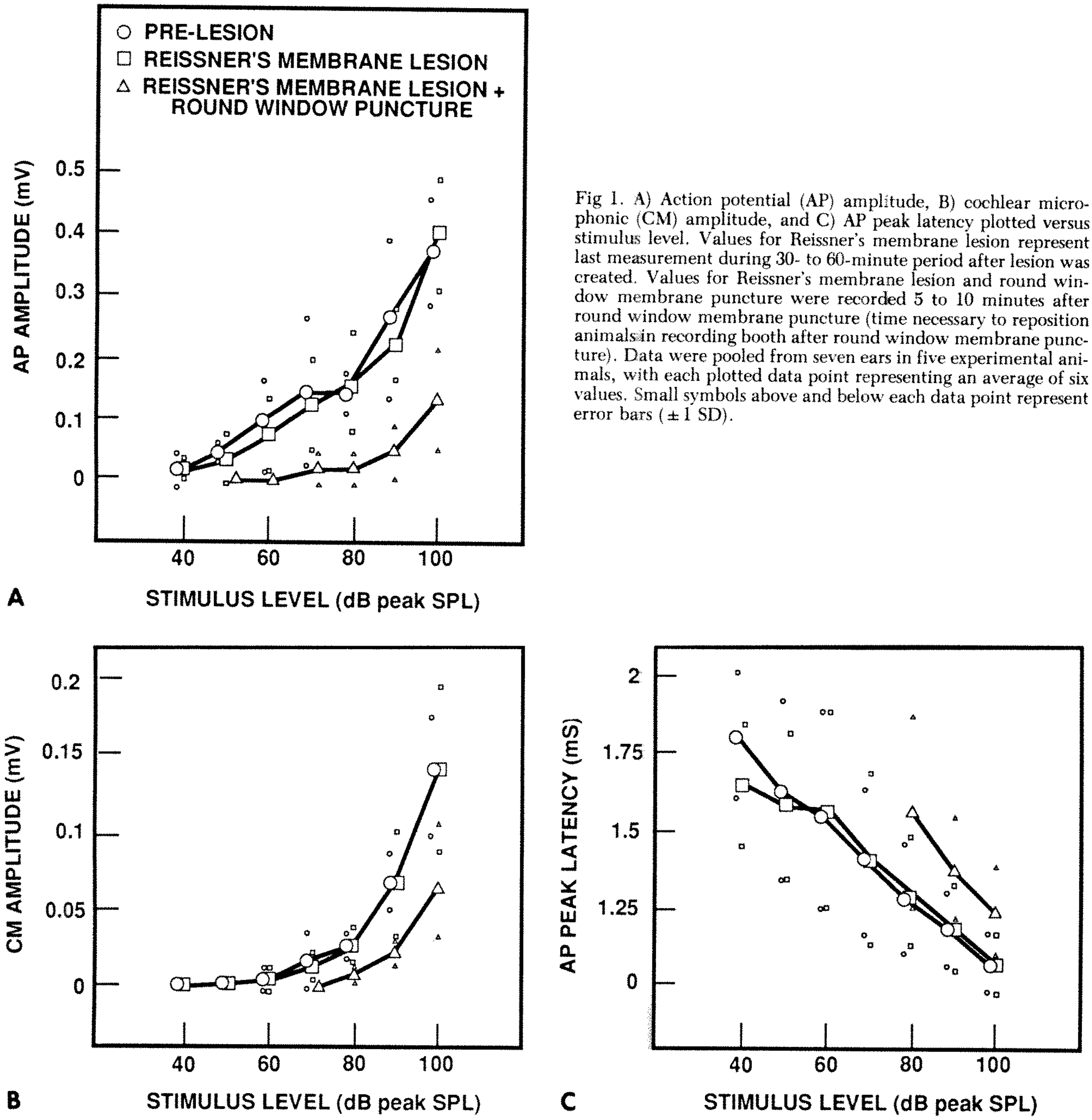


Fig 1. A) Action potential (AP) amplitude, B) cochlear microphonic (CM) amplitude, and C) AP peak latency plotted versus stimulus level. Values for Reissner's membrane lesion represent last measurement during 30- to 60-minute period after lesion was created. Values for Reissner's membrane lesion and round window membrane puncture were recorded 5 to 10 minutes after round window membrane puncture (time necessary to reposition animals in recording booth after round window membrane puncture). Data were pooled from seven ears in five experimental animals, with each plotted data point representing an average of six values. Small symbols above and below each data point represent error bars (± 1 SD).

to 10 minutes. The averaged input-output functions demonstrate a 75% to 90% drop in N1 amplitude, a 50% to 75% drop in CM amplitude, and a shift of approximately 0.2 ms of the N1 peak latency across all stimulus intensities within 5 to 10 minutes (Fig 1). This 5- to 10-minute period represents the time required to reposition the animals in the sound booth after creating the RW puncture. Measurements taken later on, typically at 1 hour, showed continued loss of responses in most cases, often to complete loss of the N1 and a slight residual CM.

Control animals with RW perforations alone did not show these decrements. In two of the six control animals there was no detectable change in the AP response up to 2 hours after RW puncture. Four of these control animals showed a gradual drop in N1 amplitude to approximately 25% of baseline after 1 hour. Representative AP waveform tracings demonstrate qualitatively the difference seen between animals with both Reissner's membrane and RW membrane lesions and control animals (Figs 2 and 3).

DISCUSSION

These results corroborate the findings of previous investigators that discrete lesions in Reissner's membrane result in only localized deficits in cochlear function. In addition to the previous findings, however, the current experiments suggest that such localization can be modified radically by a con-

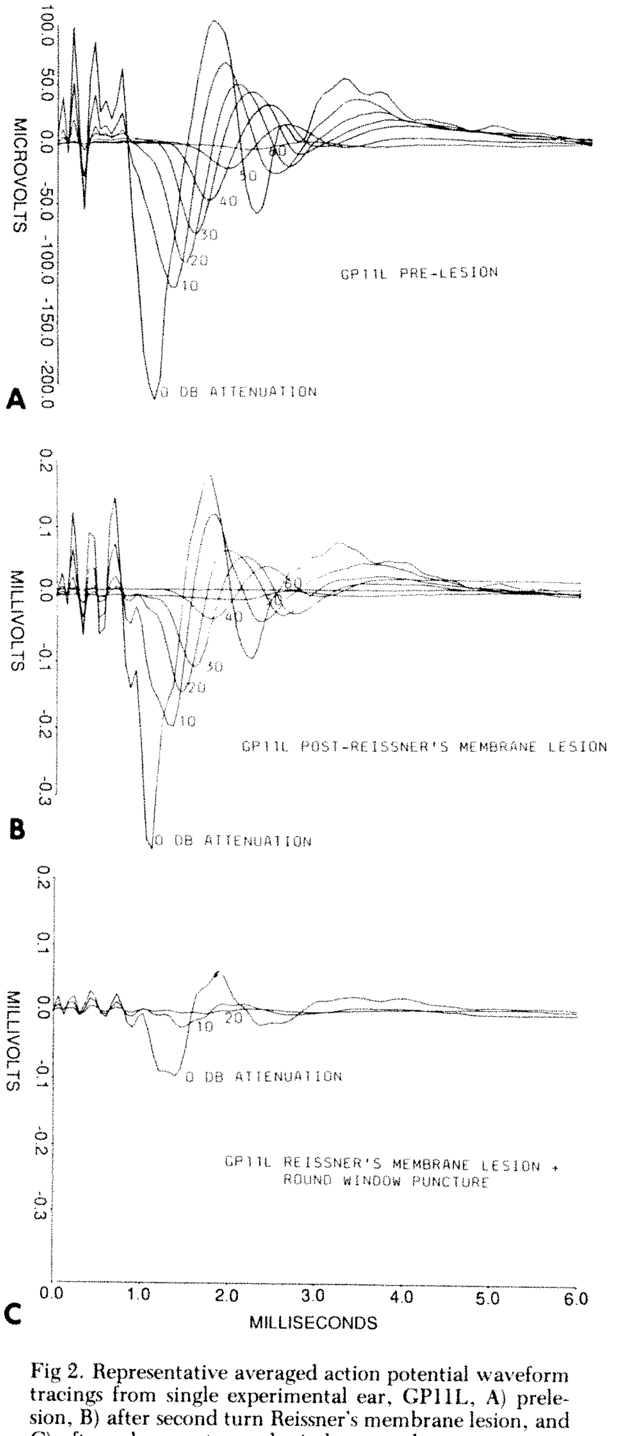


Fig 2. Representative averaged action potential waveform tracings from single experimental ear, GP11L, A) prelesion, B) after second turn Reissner's membrane lesion, and C) after subsequent round window membrane puncture.

comitant opening in the RW membrane. These experiments take advantage of the fact that the click-evoked N1 and CM recorded at the RW would not normally reflect damage to the second turn, except possibly as minor changes in the N1 waveform at low stimulus intensities.[17,18] These potentials drop dramatically only when there is cochlear damage in the basal turn and, in particular, the lower half of the basal turn. Therefore, we conclude that discrete lesions in the cochlear duct apical to the RW produce only restricted cochlear dysfunction, while in

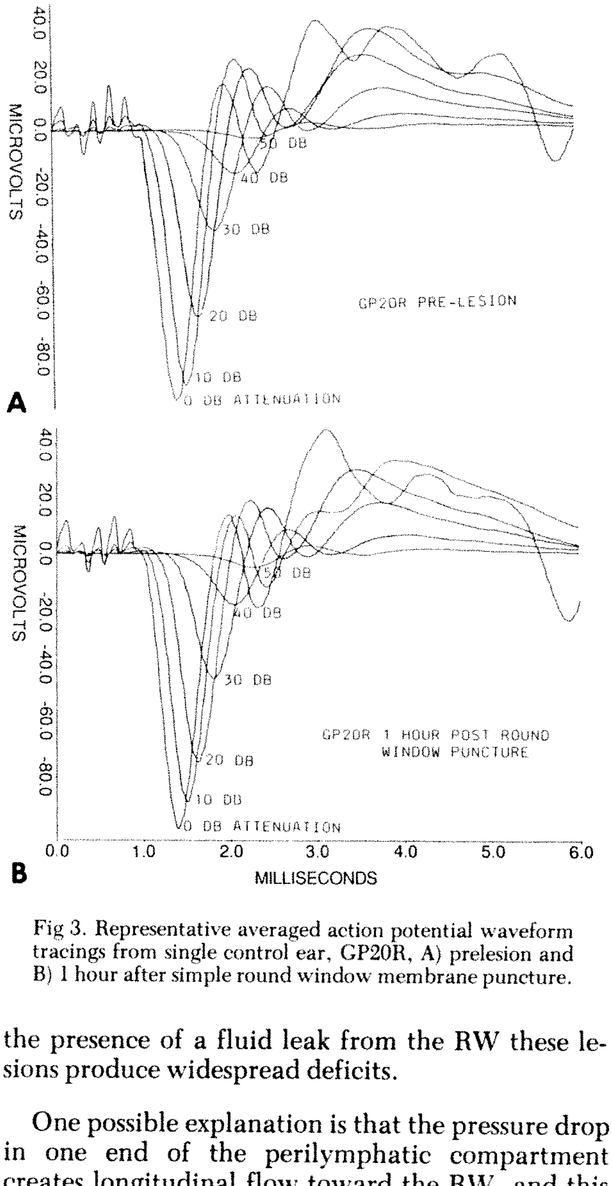


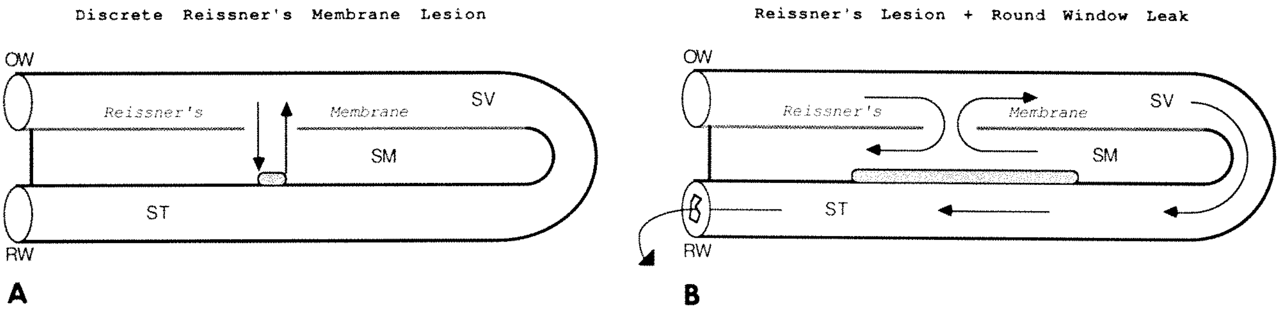
Fig 3. Representative averaged action potential waveform tracings from single control ear, GP20R, A) prelesion and B) 1 hour after simple round window membrane puncture.

the presence of a fluid leak from the RW these lesions produce widespread deficits.

One possible explanation is that the pressure drop in one end of the perilymphatic compartment creates longitudinal flow toward the RW, and this longitudinal flow results in more widespread distribution of fluid admixture (Fig 4). The experiments presented here, of course, do not provide evidence that such bulk fluid movement actually occurs in vivo. However, it is clear that in the presence of a Reissner's membrane fenestra the drop in perilymph pressure caused by the experimental PLF is sufficient to induce widespread cochlear deficits.

As cited in the Introduction, several lines of evidence, including clinical data, animal studies, and mathematical models, suggest that an isolated perforation of the RW cannot adequately explain sudden and severe cochlear dysfunction. The controls presented here likewise demonstrate that the electrocochlear response is relatively stable after RW perforation.

The only contrary data we know of are presented by Lamm et al,[19] who found RW perforation to result in a rapid 80% to 100% decrement in elec-


Discrete Reissner's Membrane Lesion
OW
Reissner's
Membrane
SV
SM
ST
RW
A
Reissner's Lesion + Round Window Leak
OW
Reissner's
Membrane
SV
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ST
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Fig 4. Proposed mechanism to explain experimental results. A) Fluid admixture remains localized with discrete Reissner's membrane lesion alone. B) With additional perilymph leak, longitudinal flow toward round window (RW) is induced, resulting in widespread fluid admixture and deficits. OW — oval window, SV — scala vestibuli, SM — scala media, ST — scala tympani.

trocochlear response in acute preparations, with complete recovery by 7 days in chronic preparations.[20] A similar study performed by Simmons et al[21] demonstrated a slow decrement in CM responses to a maximum loss of 35 to 40 dB. They documented that the majority of this loss was not reflecting cochlear damage, but was the result of fluid collecting around the recording electrode and later in the middle ear. Accordingly, there was complete recovery of hearing and electrical activity within 4 weeks. Indeed, the weight of evidence indicates not only that a RW perforation alone does not cause sudden, severe cochlear deficits, but in addition that the RW membrane is capable of repair without residual cochlear damage from the initial insult.

In light of this experimental evidence, it is difficult to imagine that PLFs alone could explain the clinical patterns of hearing loss seen in SHLs. The experimental results presented here offer an animal model for an alternative theory of SHL. Although the exact mechanics of fluid movement in this model have yet to be established, we assume that induced perilymph and endolymph admixture is the mechanism of the cochlear deficits observed. In conclusion, we present these experiments as a possible model for SHL, whose relevance lies in the demonstration that a perilymph leak in itself tends to cause large deficits in cochlear function only in the presence of an underlying defect in the cochlear duct.

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SCLEROSING ORBITAL PSEUDOTUMOR: A UNIQUE CLINICOPATHOLOGIC ENTITY

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A case of orbital pseudotumor is presented that histologically showed dense fibrosis invading bone and adjacent sinuses, and failed to respond to either steroids or radiation therapy. "Orbital pseudotumor" remains a sobriquet for a variety of clinical and histopathologic entities including a monomorphous lymphocytic benign or malignant neoplasm; a polymorphous reactive inflammatory lesion; and a densely fibrosing sclerotic variant that appears to behave more aggressively, often locally invades adjacent structures, and may be related to a multifocal fibrosclerosis that also includes retroperitoneal fibrosis, Riedel's sclerosing thyroiditis, mediastinal fibrosis, and sclerosing cholangitis. This "sclerosing orbital pseudotumor" is felt to represent a unique clinicopathologic entity, and an approach to its treatment is described.

KEY WORDS — orbital diseases.

INTRODUCTION

Orbital pseudotumor represents a poorly understood group of inflammatory infiltrates of the orbit. As our ability to characterize specific disease processes has improved, many conditions previously included under the sobriquet "pseudotumor" have been defined individually. Nonetheless, several apparently different processes continue to be grouped together under this title. More specifically, there have been several recent reports of locally aggressive pseudotumors invading bone, paranasal sinuses, dura, and brain, all of which have histologically shown dense fibrosis and have been called a sclerosing variant of pseudotumor.[1-5] A case of sclerosing orbital pseudotumor was seen recently at the University of North Carolina that progressed despite steroid and radiation therapy, resulting in a painful, fixed, blind eye requiring exenteration. Sclerosing orbital pseudotumor seems to represent a unique clinical entity, separable from other "pseudotumors," and appears to have an immunologic basis, though its cause is understood poorly. It may represent a form of multifocal fibrosclerosis, a disease that also includes retroperitoneal fibrosis, mediastinal fibrosis, Riedel's sclerosing thyroiditis, and sclerosing cholangitis.

CASE REPORT

A 24-year-old man presented to the North Carolina Memorial Hospital with a 2-week history of progressive right frontal headache, right orbital pain, and right-sided visual loss. Physical examination revealed a nontoxic-appearing young man with a temperature of 37°C. Examination of his eyes revealed a visual acuity of 20/20 in the left eye and 20/100 in the right eye. There was mild lid edema, ptosis, proptosis, and limitation of extraocular movements in the right eye. Pupils were round and reactive bilaterally. Visual field examination revealed a mild inferior temporal quadrant defect in the right eye, while tonometry revealed normal intraocular pressure of 14 mm Hg bilaterally. Funduscopic examination in the right eye revealed blurring of the disc margins with edema of the optic nerve head. Examination of the nose revealed enlarged right and left middle and inferior turbinates without purulent drainage. The oral cavity, oropharynx, nasopharynx, hypopharynx, larynx, and both sides of the neck appeared normal. The remainder of the physical examination was unremarkable. Orbital ultrasonography revealed enlargement of the right optic nerve sheath and right medial rectus muscle with orbital edema. Sinus films showed opacification of the right ethmoid sinus. The WBC count was 4,900 with a normal differential. The ESR was 13 and serum electrolytes, including serum calcium, were normal. A computed tomographic scan of the head and orbits was obtained with 5-mm cuts that revealed a soft tissue density in the superomedial portion of the right orbit extending into the right ethmoid sinus, and a right supraorbital ethmoid sinus (Fig 1A). There was enlargement of the optic nerve and muscles of the right eye. The findings were felt to be most consistent with a primary sinus infection extending into the right orbit.

The patient was admitted to the hospital and underwent an emergent right-sided external sphenoethmoidectomy with exploration of the right orbit for presumed orbital cellulitis/abscess secondary to sinus infection. At the time of exploration, thickened mucosa was found throughout the eth-

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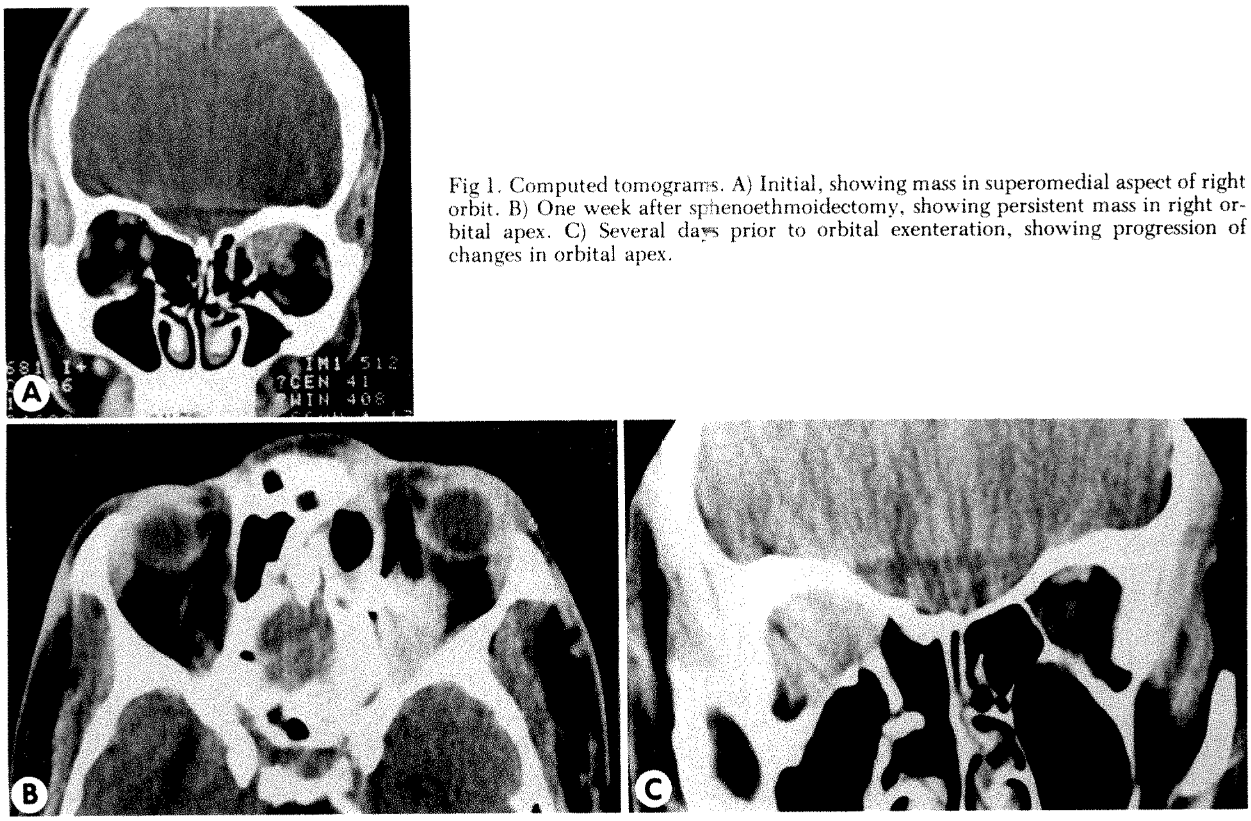

Fig 1. Computed tomograms. A) Initial, showing mass in superomedial aspect of right orbit. B) One week after sphenoethmoidectomy, showing persistent mass in right orbital apex. C) Several days prior to orbital exenteration, showing progression of changes in orbital apex.

moid sinuses. The sphenoid sinus appeared normal, as did the nasofrontal duct. A small amount of opalescent fluid was discovered in the posterior ethmoid cells. Dense white fibrous tissue was discovered in the supraorbital ethmoid cell. An incision was made into the periorbita of the right eye and this revealed only dense, thick, white fibrotic tissue. A hemostat was spread in the wound and no pus was discovered, but neither was normal-appearing orbital fat. Tissue was taken from all regions for culture and pathologic study. Cultures of the material grew rare *Propionibacterium* species and rare coagulase-negative staphylococci, both of which are susceptible to virtually all antibiotics tested. Results of fungal cultures were negative, as were those of acid-fast bacillus cultures. Histopathologic studies revealed fibrosis and acute and chronic inflammation without evidence of tumor. The fibrotic process was seen to invade bone, and the inflammatory infiltrate was polymorphic, hence inconsistent with lymphoma (Fig 2).

Postoperatively, the patient showed little change over the first few days, and orbital pseudotumor was considered as a possible diagnosis. For this reason, the patient was given oral prednisone, 100 mg per day, in addition to the chloramphenicol and oxacillin that he had been given since the operation. A repeat CT scan 1 week after sphenoethmoidectomy revealed a persistent mass in the right orbital apex (Fig 1B). On steroid therapy his pain and vision improved rapidly and returned to 20/20 in the right eye on the fourth postoperative day. The chloramphenicol was discontinued when culture sensitivities were known and the patient was continued on oxacillin for 10 days and then oral dicloxacillin for 1 month. The results of antinuclear antibody, thyroid function, and angiotensin converting enzyme tests were normal and the FTA-ABS test was nonreactive. The patient continued to take oral steroids at home (80 mg of prednisone per day). Despite this therapy, he suffered recurrent headaches, orbital pain, and decreased vision with documented visual field loss inferiorly.

A repeat CT scan done 3 weeks postoperatively revealed no change in the orbital apex mass.

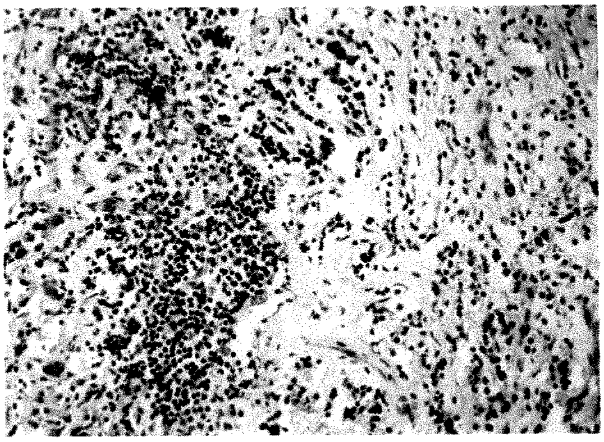

Fig 2. Initial biopsy specimen of ethmoid prior to irradiation and steroid administration, showing prominent inflammatory infiltrate composed of lymphocytes and plasma cells, with fibrosis (H & E, original x250).

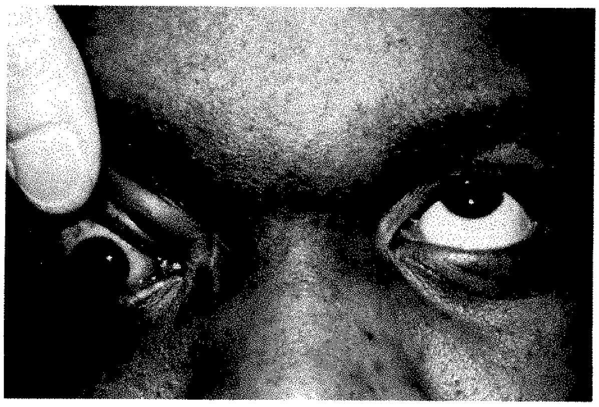

Fig 3. Patient with painful, blind, fixed right eye.

Because of his continued symptoms and especially his complaint of severe orbital pain, the patient received 2,400 rad of external beam radiotherapy in ten fractions to the right orbit and 2,000 rad of external beam radiotherapy in ten fractions to the right ethmoid over a 2-week period. During this treatment there was initially a significant decrease in his proptosis and pain, and improvement in his vision with resolution of optic nerve edema and stabilization of his visual fields. He was continued on a regimen of oral prednisone, 100 mg per day, during this time. One month after completing his radiation therapy, however, the patient had recurrent severe pain in the right orbit and a marked decrease in his visual acuity to discrimination only of hand movements in the right eye. He was admitted to the hospital and reexploration and biopsy with medial decompression of the right orbit was carried out. Biopsies revealed very dense and relatively acellular fibrous tissue with little active inflammation. The patient was discharged on the third postoperative day unchanged from his preoperative status with regard to pain or vision.

Approximately 3 months after his initial presentation, his condition progressed until he was left with a severely painful, blind, fixed, useless right eye (Figs 1C and 3). At this point, a right orbital exenteration was performed to relieve the patient of his unremitting pain and to make a definite diagnosis. At the time of operation, dense fibrous tissue was found to fill the orbital apex completely and extend into a large supraorbital ethmoid cell. All gross disease was removed with little bleeding at the time of surgery. The lid tissue was spared as the process seemed to be distant from this. Postoperatively, the patient's pain was improved greatly and he has been able to return to a productive existence. A magnetic resonance scan done 2 months after exenteration revealed no abnormality in the region of the optic chiasm and no further spread of the process.

PATHOLOGIC FINDINGS

A horizontal section through the specimen showed a normal-appearing globe with dense fibrosis of the surrounding tissues, more prominent posteriorly with encasement of the optic nerve (Fig 4A). Histologically, the globe was remarkable for an acute infarct of the optic nerve head with edema. Peripheral retinal atrophy and congestion of the choroid with a normal cornea, lens, iris, and anterior chamber angle were seen. A diffuse inflammatory infiltrate consisting of lymphocytes, plasma cells, neutrophils, and occasional macrophages and eosinophils was seen involving the orbital adipose tissue, extraocular muscles, and lacrimal gland. Prominent fibrosis with slight to marked inflammatory infiltrate was seen in the orbital apex. Small vessels showed chronic arteritis without fibrinoid necrosis of the vessel walls, while extraocular muscles showed muscle fiber necrosis with fibrosis of the endomysium. Peripheral nerves were entrapped in the fibrous tissue and revealed axonal degeneration without direct involvement with the inflammatory process. The optic nerve was surrounded and compressed by dense fibrous tissue merging with the dura mater with loss of the subarachnoid space. Focal infarcts of the optic nerve and chronic inflammation of the leptomeninges were present, while fragmentation of myelin demonstrated optic atrophy. This process extended to the cut end of the optic nerve (Fig 4B). Loose to dense chronically inflamed connective tissue with considerable fibrosis was present in the supraorbital ethmoid (Fig 4C). Immunocytochemistry revealed a polyclonal B cell proliferation staining for kappa and lambda light chains consistent with an inflammatory, non-neoplastic infiltrate. The presence of prominent fibrosis in the initial biopsy samples, which were of necessity limited in their scope; the low dose of irradiation employed; and the short period of time (2 months) between the radiotherapy and the exenteration support the view that fibrosis was an integral component of this disease process, and not simply a reaction to the radiotherapy.

DISCUSSION

The term orbital pseudotumor has been used to describe a diverse group of disease processes characterized by a mass effect of unclear causation within the orbital contents. As with so many entities in medicine, as our ability to define and characterize these processes has increased, heretofore broadly grouped diseases find specific characterization. Nonetheless, as diseases such as Graves's ophthalmopathy, Wegener's granulomatosis, polyarteritis nodosa, other vasculitides, sarcoidosis, and amyloidosis have been individually defined and cleaved off from the broader category of orbital pseudotumors, the term pseudotumor has persisted, no doubt as a conglomerate of still poorly understood disease processes.

Orbital pseudotumor is not an uncommon lesion, accounting for 13% of cases of unilateral exophthalmos, ranking second only to Graves's disease as a cause for this condition. The clinical presentation

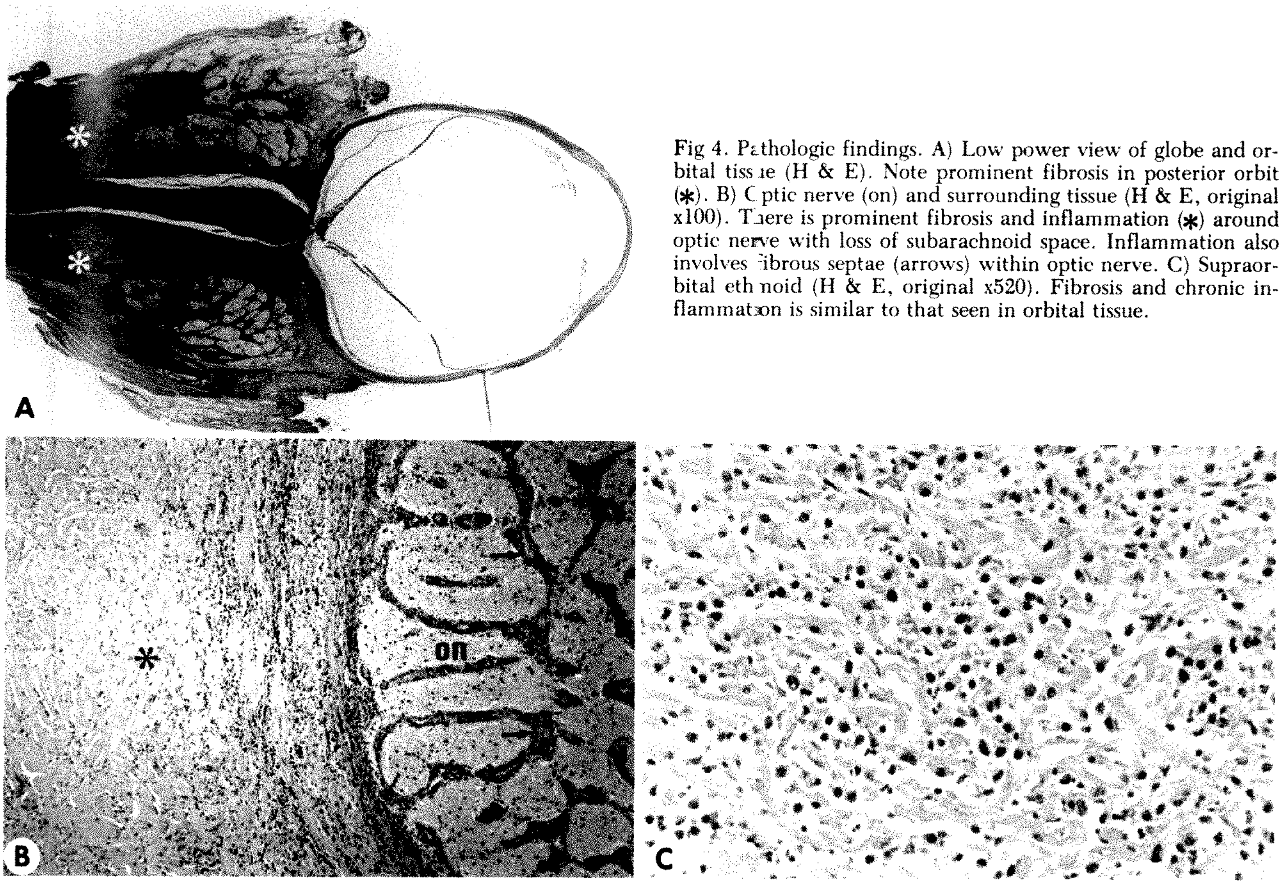

Fig 4. Pathologic findings. A) Low power view of globe and orbital tissue (H & E). Note prominent fibrosis in posterior orbit (✱). B) Optic nerve (on) and surrounding tissue (H & E, original x100). There is prominent fibrosis and inflammation (✱) around optic nerve with loss of subarachnoid space. Inflammation also involves fibrous septae (arrows) within optic nerve. C) Supraorbital ethmoid (H & E, original x520). Fibrosis and chronic inflammation is similar to that seen in orbital tissue.

is difficult to typify since most studies include a variety of lesions. In general, males and females are affected equally, with no racial predilection. The condition tends to be unilateral (more frequently bilateral in children) and usually affects persons in the seventh and eighth decades of life. It usually arises over months with exophthalmos, swollen lids, pain, limitation of extraocular movements, and visual loss,[6,7] though acute forms of presentation do occur and are usually sensitive to steroids.[8] The sclerosing, invasive form seems to follow a more indolent, chronic course.[2,4] Spontaneous regression, multiple recurrences, and steady progression with increase in proptosis and visual loss have all been described, perhaps representing different disease processes rather than variability of a single disease process. Mild CSF pleocytosis and protein elevation with signs of meningeal irritation have been described.[4] Tests for specific immunologic markers and nonspecific indicators of inflammation including antinuclear antibody levels, rheumatoid factor, ESR, WBC, eosinophilia, and elevated gamma globulins have yielded abnormal results in some but not most cases.[9]

Henderson and Farrow[6] define the orbital pseudotumor broadly as "an idiopathic, localized inflammatory disease consisting principally of a lymphocytic infiltration associated with a polymorphous cellular response and a fibrovascular tissue reaction that has a variable but self-limited course." Within this broader group they describe three subtypes: 1) a predominantly lymphoid infiltrative type, 2) a predominantly fibrotic type, and 3) a mixed variety. The classifications of others by site of involvement into dacryoadenitis, myositis, periscleritis, trochleitis, and perineuritis were based largely on limited biopsies of often more generalized disease.[6]

Jakobiec and Jones,[7] within the broad group they call idiopathic inflammatory pseudotumor, separate chronic nonspecific polymorphous orbital inflammations from the monomorphous reactive lymphocytic hyperplasias. They warn that a prominent polymorphonuclear and eosinophilic infiltrate should raise suspicions of a vasculitis. They view dense collagenization as an end stage of a disease continuum, with the earlier stages showing a greater lymphocytic component while the more advanced cases show dense collagenization. Others, such as Henderson and Farrow,[6] view the densely sclerosing types as a separate subgroup. It seems unlikely that once the process of dense hyalinization sets in, anything can be done to reverse it.

It appears that the sclerosing variant of orbital pseudotumor represents a locally aggressive entity, occurring more frequently at the orbital apex, that perhaps should be separated from the other, less virulent forms of pseudotumor. Most of the true "pseudotumors" have been felt to represent some form of immunologically mediated inflammatory response to an unknown and perhaps variable group of antigenic stimulants. This may be some form of immune complex, or cytotoxic hypersensitivity; or some form of autoimmunity. In our particular case, some immunologic stimulus from preexisting sinusitis may well have instigated the process. Orbital pseudotumor has been reported along with myasthenia gravis,[10] Crohn's disease,[11] and Still's disease,[12] all of which are felt to have an immunologic cause. On the other hand, some of the monomorphous "pseudotumors" that recently have been separated from the group as a whole may represent true neoplasms, benign or malignant. In the future more widespread use of monoclonal antibody studies should help to clarify these issues.[13]

Review of the recent literature reveals several reports of locally aggressive pseudotumors with bone erosion and sinus, dural, and brain involvement.[1-5] The majority of these cases appear to involve the sclerosing variant of orbital pseudotumor. Abramovitz et al[1] have made a case for separating the sclerosing variant from other forms of orbital pseudotumor. This variety appears to occur more frequently in the orbital apex. They, and others, feel that it may represent a variant of multifocal fibrosclerosis that also includes retroperitoneal fibrosis, Riedel's sclerosing thyroiditis, mediastinal fibrosis, and sclerosing cholangitis, with which it has been associated and which again are felt to represent some form of immunologically mediated disease process.[14-17]

The sclerosing variant has accounted for 8% to 51%[1,6] of all orbital pseudotumors, usually but not always appears to involve the orbital apex, and occurs in a younger age group, most patients presenting in the third to sixth decades. Tests of specific immunologic markers and nonspecific indicators of inflammation have not been reported widely, but results of such tests were not elevated in our patient.

Treatment has included steroids, radiotherapy, immunotherapy, and surgery. Chavis et al[18] reported that 28% of pseudotumors resolved spontaneously, 35% showed some response to steroids, and 37% were refractory to steroid therapy. None of their cases, however, were of the sclerosing variety. They found germinal follicles to be associated with spontaneous resolution and diffuse lymphocytic and lymphoblastic infiltration to be associated with a poor steroid response. Orbital apex lesions appear to respond poorly to steroids.

Donaldson et al[19] reported a good response to radiotherapy in all five patients they treated with 2,000 rad of radiotherapy; however, specific pathologic findings were not mentioned. Ampil and Bahrassa[20] and Sergott et al[21] each reported good results with low dose radiotherapy, but neither specifically mentioned the sclerosing variant and the study of Ampil and Bahrassa appears to include mostly true orbital lymphomas. Henderson and Farrow,[6] whose study included 18 (of 35) cases with the sclerosing variant, are wary of steroid therapy, feeling that it may cause only temporary suppression of the inflammatory process.

Surgical therapy usually is limited to biopsy. Some anterior lesions, however, including some sclerosing types, have been excised completely with good results. More often, however, the sclerosing variety shows diffuse involvement of all orbital apex tissues, such that complete excision would require sacrifice of the eye. It has been suggested that operating on pseudotumors may cause an exacerbation of the inflammatory process and visual loss; however, it is usually the most aggressive lesions that come to surgery, while milder cases are managed with steroids without biopsy. It is therefore difficult to ascribe the postoperative progression to the surgical manipulation itself.

It appears, then, that orbital pseudotumor continues to include a variety of lesions: the reactive pleomorphic inflammatory infiltrate with germinal centers that often may resolve spontaneously and probably will respond well to steroids; the monomorphous lymphocytic infiltrate that may represent a true neoplasm, probably should be evaluated with monoclonal antibody studies, and may well be treated best with radiotherapy; and the sclerosing variety that appears to respond poorly to either steroids or irradiation, may be locally aggressive, and may be related to multifocal fibrosclerosis. Tolosa Hunt syndrome is probably a clinical variant of this lesion with involvement of the superior orbital fissure and dura surrounding the cavernous sinus.[2,4] It seems unlikely that this entity is merely an end stage of other forms of orbital pseudotumor. It appears to be due to an immunologic stimulus causing progressive inflammation and fibrosis of the orbital tissues. In the present case, chronic sinusitis may have been the immunologic stimulus of the process. Whether progression of this variant of the disease is preventable remains unknown; in certain cases, including our own, steroids and irradiation have provided a temporary improvement but were ineffective in preventing steady progression. The natural course of the disorder is unknown, though local aggressiveness with involvement of bone, sinus mucosa, dura, and brain has been reported.[2-5] Whether complete local excision will remove the inflammatory stimulus also is unknown and we continue to observe our patient carefully with magnetic resonance images of the optic chiasm area for any evidence of recurrent disease.

CONCLUSION

The sclerosing variant of orbital pseudotumor appears to represent a specific disease entity that may be locally aggressive and may be related to multifocal fibrosclerosis. Though its natural history is defined poorly as yet, it may progress despite therapy with steroids and irradiation and ultimately require surgical excision. Nonetheless, it appears to represent a benign immunologically mediated disorder warranting attempts at initial conservative therapy. In some cases total surgical excision has halted the local process. In any case of orbital pseudotumor, a thorough physical and laboratory search for specific causes should be performed, and if this is unproductive, an initial trial of steroid therapy is indicated. Cases not responding to steroids should be sampled for biopsy, with tissue saved for monoclonal antibody studies that then can be performed as the pathologist sees fit. Cases in which a predominantly lymphoid infiltrate is found may be treated with radiotherapy with expectation of some response. Cases showing extensive fibrosis have a poor prognosis and specific treatment recommendations are difficult to make at this time. If the lesion is anterior and easily encompassed surgically, preserving the eye, this would seem a reasonable approach. If, as is more commonly the case, the lesion is located posteriorly, then a trial of low dose radiotherapy is probably indicated, reserving exenteration for progressive, painful disease with a blind eye. Hopefully, as this sclerosing variant becomes more widely recognized, treatment protocols based on more information can be formulated.

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UVULOPALATOPHARYNGOPLASTY: POSTOPERATIVE MANAGEMENT AND EVALUATION OF RESULTS

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Since the earliest report of success with the uvulopalatopharyngoplasty (UPPP) operation, several authors have reported less than optimal results in their experience with the procedure. Part of the difficulty in evaluating the objective results of postoperative sleep studies has been the variations in reporting data. In addition, no standard of postoperative care, including analgesia, and immediate postoperative monitoring has been described. Our experience with 42 UPPP patients (34 studied with preoperative and postoperative polysomnography) indicates a 76% response (56% successes, and an additional 20% with limited success) with use of the more stringent criteria described recently. We discuss our management of these cases, including postoperative oxygen saturation monitoring and avoidance of narcotic analgesia as necessary factors for success with this operation.

KEY WORDS — obstructive sleep apnea, uvulopalatopharyngoplasty.

INTRODUCTION

Since Gastaut et al[1] initially reported on respiratory pauses during sleep and the Pickwickian syndrome, continued investigation has spurred our current knowledge of the obstructive sleep apnea (OSA) syndrome. Increasing medical and public awareness and the availability of sleep monitoring techniques, especially polysomnography (PSG), are leading to the increased frequency of presentation of these patients for definitive surgical care.

Obstructive sleep apnea is a disorder characterized by a wide range of severity of subjective symptoms including but not limited to daytime hypersomnolence, headache, altered mentation, and snoring. Objective measurement, using PSG, documents episodes of apnea and desaturation that are often more severe than one would expect from a patient's subjective complaints.

Medical management of the disorder has been unpredictable. While nasal continuous positive airway pressure (CPAP) has shown promise as a nonsurgical approach to treatment, it has met with limited acceptance. Until recently, tracheotomy was and is still considered by some the only effective treatment. The adverse social, psychological, and physical effects of permanent tracheotomy have been described[2] and are familiar to physicians treating these patients. In an effort to obviate the need for tracheotomy, Fujita et al[3] developed the uvulopalatopharyngoplasty (UPPP) operation. In 1981 they described initial success in alleviating the symptoms of obstructive sleep apnea in 12 patients. Since that report, the procedure, designed to alleviate the anatomic alterations that lead to obstruction of the velopharyngeal/oropharyngeal space, gradually has become accepted as a treatment for the disorder. At times tracheotomy is done in conjunction with UPPP. The reports that followed from other centers described mixed results, which can be accounted for partially by the wide range of severity of presenting symptoms, as well as varying reporting parameters. This variability in subjective and objective criteria has made postoperative PSG testing mandatory in assessing the efficacy of the UPPP procedure. Even more variable and unpredictable has been the individual response to surgery. The fact that an accepted standard of reporting results and quantifying severity has not been established has made comparison of results difficult.

Despite reports of less than favorable objective results with UPPP as the treatment of OSA,[4,5] it was our impression, after performing the operation as a primary procedure for 4 years, that our objective results were more favorable. We, as well as most investigators reporting on the UPPP operation, noted greater than 85% subjective improvement in symptoms of excessive daytime somnolence and greater than 90% improvement in snoring in all patients undergoing UPPP. Objective data, however, were not always in agreement with subjective estimations of well-being. We review our data for 34 patients who underwent UPPP and had preoperative and

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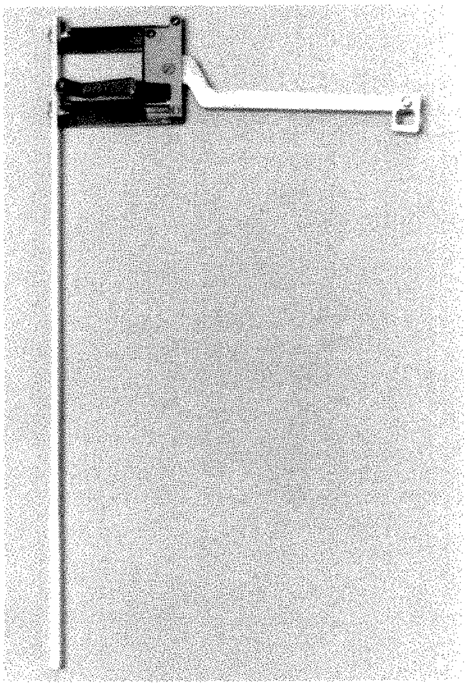

Fig 1. Custom suspension device for McIvor mouth gag that allows for adequate chest excursion in obese patient.

postoperative PSG studies, and evaluate any predictive variables for surgical outcome that to date have been elusive. Finally, we describe our evaluation, selection, and management criteria.

PATIENTS AND METHODS

Forty-two patients underwent UPPP as the primary treatment for OSA at the Geisinger Medical Center between Jan 1, 1983, and March 31, 1987. Thirty-four of these patients are included in this study, having undergone preoperative PSG and repeat study 6 weeks or longer post-UPPP. Of the 34 patients, four underwent tracheotomy as part of their treatment. All patients subsequently were decannulated. Three patients also underwent septoplasty. Eight of the initial 42 patients failed to return for postoperative testing; however, their preoperative test results were similar to those of the study group (mean apnea index, 23 versus 29 for study patients). The group consisted of 31 men and three women, with a mean age of 50 (range, 15 to 73 years) and mean weight of 113 kg (252 lb) (range, 77 to 170.3 kg [170 to 375 lb]).

TABLE 1. POLYSOMNOGRAPHY RESULTS FOR ENTIRE STUDY GROUP (MEAN ± SD; N = 34)

	*Apnea Index**	*Respiratory Disturbance Index†*	*Lowest Oxygen Saturation During Study Period*
Preoperative	29.3 ± 18.5	48.4 ± 26.2	65.4 ± 13.3
Postoperative	6.5 ± 13.9	13.5 ± 18.9	76.7 ± 15.1

*No. of apneas per hour.

†No. of apneas and hypopneas per hour.

As part of the evaluation, each patient underwent a complete history and physical examination by a pulmonary medicine specialist and a complete otolaryngic examination by an otolaryngologist, including nasopharyngoscopy with sitting and supine Mueller maneuvers. Cephalometric analysis was performed on nearly all of the group; unfortunately, a significant number of the patient records were incomplete, making interpretation of cephalometric data inconclusive. Each patient also underwent full nocturnal PSG, including EEG, electro-oculogram, and electromyogram staging of sleep, as well as ear oximetry recording of oxygen saturation, abdominal and chest wall strain gauge measurements for respiratory effort, and nasal thermistor recording of airflow. The ECG was monitored constantly and showed no serious dysrhythmia in any patient other than occasional premature ventricular contractions and sinus arrhythmia. Each PSG was evaluated by a clinical polysomnographer and pulmonologist (A.M.) and scaled according to the following criteria. Apnea was defined as any cessation of airflow for at least 10 seconds, with 30 or more episodes in a 7-hour period considered abnormal in patients under the age of 60. Hypopnea, which together with apnea makes up the respiratory disturbance count, is defined as a respiratory disturbance resulting in desaturation of >4%, or associated with awakening. The majority of our patients demonstrated obstructive or mixed apneic episodes; none had pure central apnea.

PATIENT SELECTION

All patients had histories consistent with OSA including excessive daytime somnolence and loud snoring. Uvulopalatopharyngoplasty was performed on the basis of a diagnosis of obstructive or mixed apnea on preoperative PSG, in addition to the following criteria: a positive Mueller maneuver in either position, characterized by >50% diminution of the velopharyngeal airway; characteristic findings on oral inspection of a floppy broad uvula and pharyngeal mucosal redundancy with or without tonsillar hypertrophy; and evidence on cephalometric analysis of isolated oropharyngeal obstruction. Patients demonstrating varying degrees of OSA who did not meet the criteria listed and were not responding to medical management underwent tracheotomy alone and are not included in this investigation.

Tracheotomy was performed 3 months prior to definitive UPPP in one patient who presented with cor pulmonale, and at the time of UPPP surgery in three morbidly obese patients to assure a safe airway. All UPPP patients in this study who underwent tracheotomy were decannulated eventually. Patients with normal PSG results who underwent UPPP for snoring alone were excluded from the

TABLE 2. CRITERIA FOR SUCCESS AND SUCCESS RATES

Study	*Criteria*	*Success (% of Patients)*
DeBerry-Borowiecki et al[5]	Change in AI $>50\%$, sat deficit	22% (44%)*
Katsantonis et al[6]	No. of events associated with sat $<85\%$	66%
Wetmore et al[7]	AI <5, change in RDI $>50\%$, lowest sat $>82\%$	30% (63%)*
Fujita et al[3]	Change in AI $>50\%$	50%

Note that these studies use oxygen saturation criteria as well as AI.

AI — apnea index, sat — oxygen saturation, RDI — respiratory disturbance index.

*Percentage in parentheses — improved but not "success" by criteria listed.

TABLE 3. STRATIFICATION OF PATIENTS INTO CLINICAL RESPONSE GROUPS

Group	*Apnea Index*	*Change in Respiratory Disturbance Index*	*Lowest Oxygen Saturation During Study Period*
Group A (marked improvement)	<5	$>50\%$	$>82\%$
Group B (improvement)	Change of $>50\%$		$>60\%$
Group C (failure)	Change of $<50\%$	$<50\%$	$<60\%$

Based on criteria from Wetmore et al.[7]

study, but showed nearly 100% resolution of snoring.

The UPPP was performed in the standard fashion described by Fujita et al.[3] Palatal musculature and posterior pharyngeal mucosa were not violated. Neither postoperative nasopharyngeal stenosis nor persistent nasopharyngeal regurgitation was encountered in this study, although they have both been reported as a complication of the procedure.[6] A technical modification of the procedure was the use of a customized suspension device designed by Dr F. W. Davison for tonsillectomy and modified by the first author (R.A.M.) to adapt to the usually obese OSA patient (Fig 1). This device is used in conjunction with a large blade McIvor mouth gag to provide adequate exposure of the entire operative field. This has overcome intraoperative problems with limitation of chest excursion by the Mayo stand formerly used as a hanger for the mouth gag.

RESULTS

We compared the preoperative and postoperative UPPP results of all 34 patients. Evaluation of the group as a whole showed improvement in all parameters tested (Table 1). Because of individual variability, presenting mean data of the entire group tends to minimize response or nonresponse at either end of the scale. To analyze more appropriately the broad range of response to this surgery, most investigators, including the authors, have divided the group into responders or nonresponders on the basis of individualized criteria (Table 2[3,5-7]). Using criteria outlined by Wetmore et al (Table 3),[7] 19 of 34 patients, or 56%, were successes, showing a marked improvement in parameters, with an apnea index <5, respiratory disturbance index change $>50\%$, and lowest saturation $>82\%$. These patients represent a successful outcome and are labeled group A responders. An additional seven of 34 showed a limited response, as defined by an improvement in apnea index by $>50\%$ and lowest saturation $>60\%$, and are considered to be group B or limited responders. Combining the two groups, those successes in group A, and those with a limited response in group B, derives the net combined favorable response of 76%. The remaining eight patients failed to meet the criteria for response and are considered surgical failures (group C in criteria of Wetmore et al[7]). Table 4 lists the individual PSG results in descending order of severity by respiratory disturbance index. The means for each separate group are listed in Table 5 and demonstrated schematically in the box plots in Fig 2.

DISCUSSION

We chose to use the criteria of Wetmore et al[7] of response to surgery (Table 3) because they take into account several parameters: apnea index, respiratory disturbance index (the number of apneic episodes per sleep hour, combined with hypopneas), and lowest saturation (included as an approximation of desaturation changes).

The importance of using a saturation parameter when defining success or failure of this procedure becomes apparent when one examines the box plots in Fig 2, which demonstrate a significant improvement in mean apnea index and respiratory disturbance index for all groups. Improvement in mean oxygen saturation is seen only in group A patients. Groups B and C exhibit virtually no change in this parameter. It seems imperative, in light of this and the fact that oxygen desaturation is associated with potentially fatal cardiac arrhythmias, that we define success in terms of several parameters, including the changes in saturation. If we use the criteria of Fujita et al[3] of apnea index improvement of $>50\%$ to define success in our patient population, a rate of 85% was attained. Using the criteria of Wetmore et al[7] results in a 56% success rate, which, while smaller and less desirable in arguing the case for UPPP, seems more accurate in defining physiologic improvement.

Determining predictive values for success from UPPP by use of preoperative and postoperative PSG results has been elusive. In our series, an increase in absolute weight exerted a statistically significant negative influence on outcome ($p = .0001$) with use of a least squares linear regression model. Fujita et al[8] found weight less than 125% of ideal as a nega-

TABLE 4. INDIVIDUAL POLYSOMNOGRAPHY DATA

		RDI			*Apnea Index*			*Lowest O2 %*			
Group	*Patient*	*Preop*	*Postop*	*% Change RDI*	*Preop*	*Postop*	*% Change AI*	*Preop*	*Postop*	*Oximeter*	*Preoperative Weight in kg (lb)*
A	22	39.710	0	100.000	26.170	0	100.00	72	83	*	90.8 (200)
A	20	40.260	0	100.000	11.440	0	100.00	58	95	*	97.2 (214)
A	16	49.430	6.996	85.840	44.570	0.152	99.65	48	88	>87%	96.2 (212)
A	11	60.000	3.918	93.460	60.000	0.890	98.51	40	81	*	94.9 (209)
A	32	14.210	1.350	90.500	6.689	0.300	95.51	81	90	>93%	94.4 (208)
A	34	9.569	2.326	75.690	5.204	0.136	97.37	73	82	*	134.8 (297)
A	21	40.200	4.984	87.600	37.400	4.980	86.67	75	85	>93%	113.5 (250)
A	31	15.150	0	100.000	9.642	0	100.00	74	95	>88%	100.8 (222)
A	30	15.710	0	100.000	12.200	0	100.00	82	90	*	91.3 (201)
A	28	20.970	2.932	86.020	20.100	1.800	91.02	60	87	>92%	117.1 (258)
A	33	9.900	0	100.000	9.306	0	100.00	82	92	>95%	145.7 (321)
A	26	23.850	0	100.000	19.440	0	100.00	84	90	>92%	109.9 (242)
A	24	25.940	0	100.000	4.864	0	100.00	87	95	>90%	170.3 (375)
A	8	73.050	0	100.000	32.630	0	100.00	67	100	>91%	144.8 (319)
A	6	77.060	6.842	91.120	57.330	2.630	95.40	71	83	>90%	81.3 (179)
A	17	46.280	1.776	96.160	46.280	1.500	96.75	69	88	*	90.8 (200)
A	5	82.270	4.818	94.140	25.580	1.370	94.61	65	82	>86%	97.2 (214)
A	3	89.610	0.396	99.550	9.506	0	100.00	40	83	>75%	*
A	25	25.800	4.818	81.320	25.600	1.370	94.62	52	82	>80%	106.2 (234)
B	10	65.710	12.070	81.610	37.000	4.600	87.54	40	75	*	103.1 (229)
B	4	85.010	23.860	71.930	56.050	0	100.00	69	67	*	113.5 (250)
B	14	57.160	4.315	92.450	50.340	1.650	96.70	65	70	>70%	97.6 (215)
B	19	40.890	16.420	59.820	15.150	3.710	75.49	53	64	>89%	92.2 (203)
B	13	58.000	17.810	69.280	33.890	6.540	80.69	53	63	>93%	109.0 (240)
B	27	22.800	4.615	79.750	17.650	4.390	75.10	77	73	>93%	77.2 (170)
B	15	51.970	5.020	90.340	27.270	0	100.00	71	68	*	118.0 (260)
C	7	77.050	63.760	17.240	26.620	62.900	−136.00	73	75	*	145.3 (320)
C	12	59.190	53.890	8.954	6.807	7.040	− 3.44	66	60	*	106.2 (234)
C	18	41.700	41.860	0.394	18.150	1.760	90.25	68	56	*	156.6 (345)
C	1	96.010	44.110	54.050	69.890	8.510	87.82	56	43	>90%†	157.5 (347)
C	23	38.000	32.200	15.240	29.780	28.60	3.96	64	72	>93%	91.7 (202)
C	2	95.960	25.690	73.220	31.400	18.70	40.13	48	67	>93%†	163.4 (360)
C	9	67.640	62.710	7.294	66.620	31.60	52.43	84	34	>93%	158.9 (350)
C	29	17.140	8.285	51.660	15.000	8.280	44.76	60	70	*	*

RDI — respiratory disturbance index, lowest O2% — lowest oxygen saturation on formal sleep studies done preoperatively and approximately 6 weeks postoperatively, oximeter — lowest oxygen saturation recorded in immediate postoperative period.

*Information not available.

†Required oxygen therapy postoperatively.

tive predictor, but this has not been found by others, who advocate the procedure for less obese patients.[9] Conway et al[10] further stated that maintenance of body weight assures continued benefit from UPPP. Increasing age, absolute number of apneas, and total number of respiratory disturbances also statistically predicted a negative outcome. While postoperative PSG measurements of lowest oxygen saturation are indicative of disease severity and certainly are an important factor in the development of cardiac arrhythmia, the preoperative PSG parameter of lowest oxygen saturation was not able to predict success or failure statistically.

One single factor in the initial evaluation that seems to have significance in determining improvement is the anatomic level of obstruction. Several investigators who feel UPPP has a limited usefulness for OSA are not selective as to site of obstruction in their patients.[5,9,11] Simmons et al[9] were not able to predict success or failure of the UPPP operation on the basis of the anatomic appearance of the oropharynx alone. We limit our procedure to those patients demonstrating only oropharyngeal obstruction that can be documented on oral inspection and with supine and sitting fiberoptic nasopharyngoscopy (Mueller maneuver). Sher et al[12] described the high predictive value of the Mueller maneuver in patient selection, and obtained similar good results by eliminating as potential nonresponders those patients who did not demonstrate at least a 50% closure of the soft tissues of the velopharyngeal area. Seventy-three percent of their study patients demonstrating a greater than 50% closure of the nasopharyngeal airway with forced inspiration against an occluded airway had a 50% improvement in respiratory disturbance index. Cephalometric studies have been questioned as a suitable preoperative analysis, since one obtains only midline information. A new procedure by Crumley et al,[13] cine-computed tomography, holds promise in evaluating site of obstruction, but its cost and specialized equipment demands may relegate it to research use only.

TABLE 5. POLYSOMNOGRAPHY DATA BY GROUP (MEAN±SD)

		Apnea Index	*Respiratory Disturbance Index*	*Lowest Oxygen Saturation*
Group A (n = 19)	Preop	24.4±17.6	39.94±25.7	67.3±14.3
	Postop	0.8± 1.3	2.16± 2.5	88.0± 5.6
Group B (n = 7)	Preop	33.9±15.4	54.5 ±19.5	61.1±13.0
	Postop	3.0± 2.5	12.0 ± 7.7	68.7± 4.4
Group C (n = 8)	Preop	33.0±23.2	61.6 ±28.2	64.8±10.8
	Postop	21.2±20.0	41.5 ±19.1	59.6±14.6

Criteria for groups were defined by Wetmore et al.[7]

Since 1983, we have performed UPPP as the primary management of OSA, without regard to the degree of severity of disease demonstrated by preoperative PSG, when the above criteria of anatomic obstruction are met. Despite reports in the literature that advocate tracheotomy as the only reliable method to treat OSA,[4,11] it is our impression that UPPP can be used as the initial therapeutic modality for patients presenting with all degrees of OSA, with tracheotomy reserved for failures. Interestingly, Caldarelli et al[14] recommended UPPP for only those patients demonstrating severe forms of OSA, with an apnea index >70.

To lessen complications, several important management points should be followed. Since 1986, we have adopted a policy of avoiding narcotics or sedatives in the postoperative period. We have relied on acetaminophen as our only analgesic agent. This policy was adopted after one of our patients became obtunded after 60 mg of codeine was administered orally and subsequently developed adult respiratory distress syndrome from presumed aspiration. This patient, who had one of our most severe cases of OSA, went on to full recovery and is classified a group A responder. We counsel all patients preoperatively on the use of no narcotics, and have had no serious difficulties with patient compliance. We have just begun to use an oral anesthetic (Chloraseptic) on a limited number of patients and find it a valuable adjunct.

Additionally, we have standardized our postoperative routine. Each patient is admitted to the special care unit (an intensive care stepdown unit) the first night postoperatively. Continuous ECG monitoring is performed, along with continuous nocturnal ear oximeter tracings. Cool mist room air is prescribed and is supplemented with oxygen if saturation drops below the 80% to 85% range for more than a few minutes. In this way those patients with a tendency to desaturate are identified routinely and arrangements are made for earlier postoperative PSG and appropriate therapy, usually

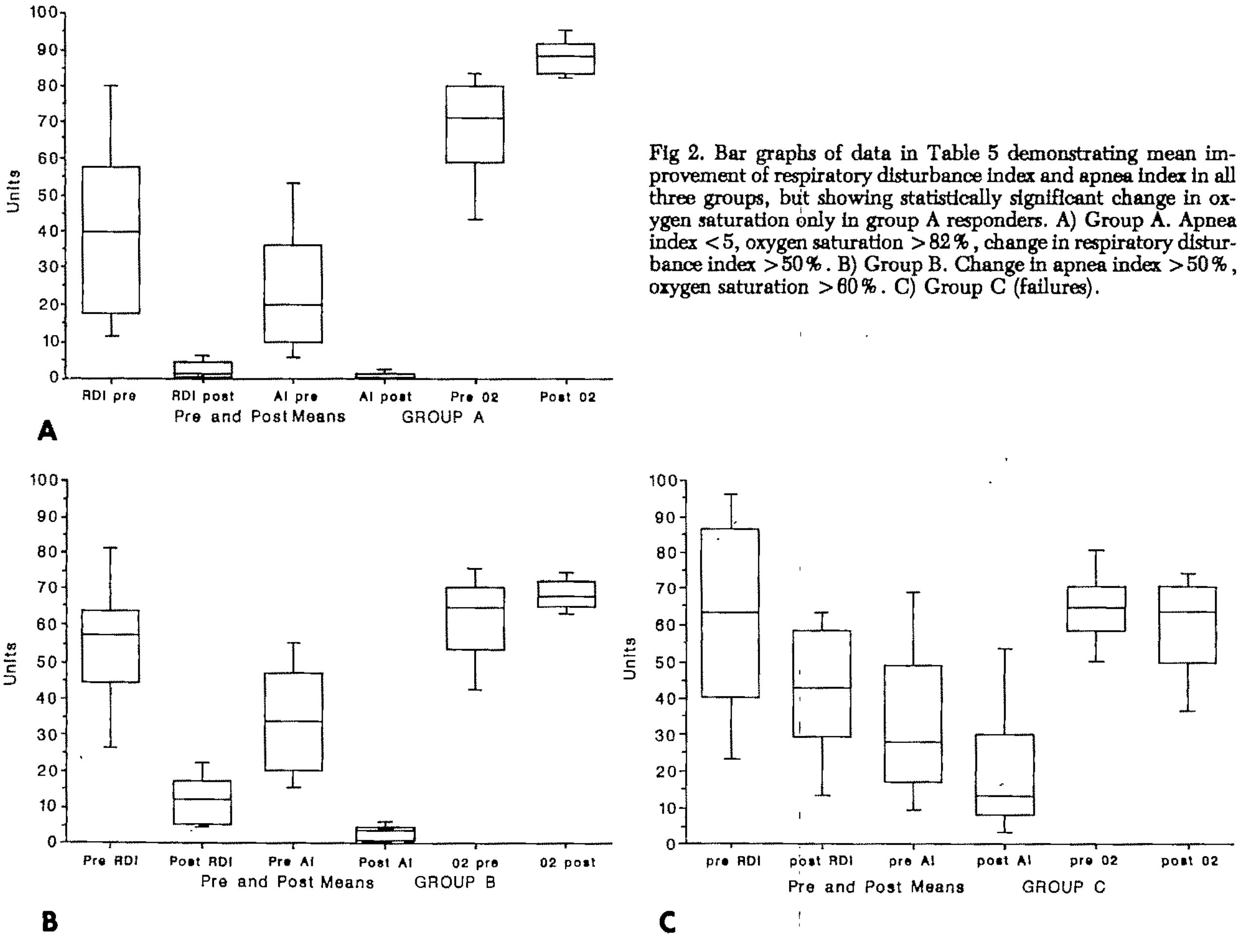

Fig 2. Bar graphs of data in Table 5 demonstrating mean improvement of respiratory disturbance index and apnea index in all three groups, but showing statistically significant change in oxygen saturation only in group A responders. A) Group A. Apnea index <5, oxygen saturation >82%, change in respiratory disturbance index >50%. B) Group B. Change in apnea index >50%, oxygen saturation >60%. C) Group C (failures).

tracheotomy. The three patients in our study who required oxygen postoperatively to maintain their saturation above 80% are all group C failures (Table 4). This fact suggests that those who are going to fail UPPP manifest early; however, some patients in the study were seen prior to institution of ear oximeter monitoring, and statistical significance for this observation was not identified. The use of routine saturation monitoring may serve to avert discharging severe failures, which can lead to disastrous consequences.[15] Despite reports of substantial worsening of saturation in the immediate postoperative period,[15] we have not experienced such, probably in part because of our policy of no narcotic analgesia and possibly because of general anesthetic effect, which may differ among institutions.

After the first night in the special care unit, patients demonstrating adequate saturation are transferred to the general otolaryngology ward and oximetry is discontinued. Patients are encouraged to take oral feedings, and most are discharged on the third postoperative day.

CONCLUSION

In summary, we have performed UPPP as a primary procedure for OSA in patients with obstruction at the level of the velopharyngeal airway. Our success on the basis of several criteria supports the procedure as a first-line surgical management of all stages of OSA. Tracheotomy is reserved for failures or those who cannot undergo the procedure because of serious medical problems. Change in apnea index alone is not sufficient to determine success with the procedure. Saturation scores also should be used. Increasing age, weight, and number of respiratory disturbances all had a negative effect on outcome. A policy of avoiding sedatives or narcotics, and routine postoperative oximetry monitoring in a special care unit, will lead to fewer complications and better results in this group of patients. We echo the recommendation of others that it is imperative to have adequate follow-up studies to assure that persistent disease does not go untreated.

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ANALOG AND DIGITAL FILTERING OF THE BRAIN STEM AUDITORY EVOKED RESPONSE

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This study compared the filtering effects on the auditory evoked potential of zero and standard phase shift digital filters (the former was a mathematical approximation of a standard Butterworth filter). Conventional filters were found to decrease the height of the evoked response in the majority of waveforms compared to zero phase shift filters. A 36-dB/octave zero phase shift high pass filter with a cutoff frequency of 100 Hz produced a 16% reduction in wave amplitude compared to the unfiltered control. A 36-dB/octave, 100-Hz standard phase shift high pass filter produced a 41% reduction, and a 12-dB/octave, 150-Hz standard phase shift high pass filter produced a 38% reduction in wave amplitude compared to the unfiltered control. A decrease in the mean along with an increase in the variability of wave IV/V latency was also noted with conventional compared to zero phase shift filters. The increase in the variability of the latency measurement was due to the difficulty in waveform identification caused by the phase shift distortion of the conventional filter along with the variable decrease in wave latency caused by phase shifting responses with different spectral content. Our results indicated that a zero phase shift high pass filter of 100 Hz was the most desirable filter studied for the mitigation of spontaneous brain activity and random muscle artifact.

KEY WORDS — auditory brain stem response, auditory evoked potentials, auditory threshold testing, digital filtering.

INTRODUCTION

Significant spontaneous brain activity and random muscle artifact can distort the auditory evoked potential and make recording almost impossible. In neurodiagnosis and threshold testing it is often desirable and necessary to eliminate this artifact in order to record a clear response. The frequencies of spontaneous brain activity vary with the level of subject consciousness and overlap the response frequencies of auditory evoked potentials. The peak frequency of muscle potentials is approximately 60 Hz. This peak is broad based, with the majority of energy below 300 Hz.[1] The response frequency peaks of the early portion of the auditory evoked potential (auditory brain stem response) are located approximately at 100 Hz, 500 Hz, and 1,000 Hz.[2,3] The peak frequency of muscle artifact is less than an octave away from the frequency peak of the auditory brain stem response's slow component (100-Hz spectral peak) and there is considerable frequency overlap. Thus, a steep filter is needed in order to eliminate a substantial portion of the artifact. However, steep analog filters cause significant phase shift distortions of the auditory brain stem response.[4-6] Thus, shallow high pass filters (12 dB/octave or less) usually are used to mitigate the spontaneous brain activity and random muscle artifact. Often the cutoff frequency of the filter is raised to 150 Hz in order for these shallow filters to be effective and still allow the recording of the response.

This article will quantitate the effects of high pass analog and digital filtering on the first 15 ms of the auditory evoked potential. In contrast to previous studies, a digital filter was used with a resolution of 16.7 Hz, which was several octaves below the studied cutoff frequencies of 100 and 150 Hz.

The potentials studied in this paper were elicited by 35-dB normal hearing level (nHL) stimuli. Little research has been reported concerning the effect of filtering on auditory brain stem responses elicited by low intensity stimuli. In the more poorly defined, near-threshold waveforms (as also recorded with higher stimulus intensities in patients with hearing loss), phase shift distortions may make the identification of individual waveforms more difficult because the peaks of the high frequency component (waves I through V) are less well defined.

METHODS

Twenty-three audiometrically normal subjects were evaluated in this study. All subjects were tested in a soundproof room (Tracoustic model RS-255A) while resting in a reclining chair. No sedation was used.

The Pathfinder II (Nicolet Corp) was used to generate the signals, average the responses, and digitally filter the waveforms. The presenting stimuli were 35-dB nHL, 0.1-ms rarefaction clicks presented at a rate of 9.7/s. All responses were recorded with open filters (0.2 to 8,000 Hz) by use of a 12-dB/octave Butterworth filter. Electrodes were placed on the vertex (+), ipsilateral mastoid (−), and contralateral mastoid (ground). Electrode impedance was less than 3 kHz.

All waveforms were recorded twice with 1,000 stimulus presentations each, to allow for verification of the data. The two averages were added

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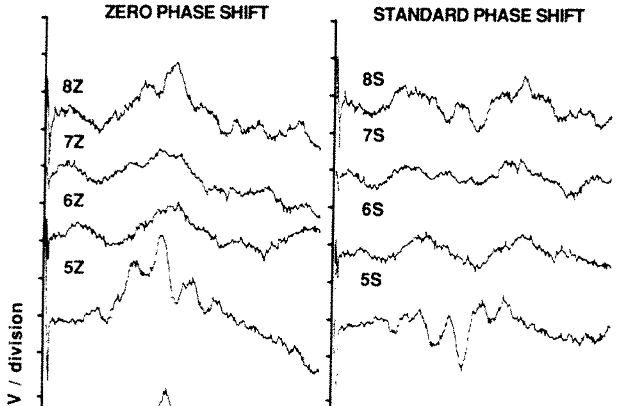

Zero and standard high pass digital filtering. High pass filter is 100 Hz, 36 dB/octave. Eight consecutively tested subjects (1 through 8) are shown.

together and baseline corrected. The waveforms, consisting of 2,000 averages each, were then digitally processed with a Fortran-based digital filter that mimicked a Butterworth filter with 12- and 36-dB/octave slopes and high pass cutoff frequencies of 100 and 150 Hz.[7] Both zero and standard phase shift conditions also were analyzed for all waveform sets. The filtering program implemented a discrete Fourier transform. The phase and power spectra were calculated for each waveform. The spectra then were weighted by use of the phase and power input/output functions of the desired filter. By use of these new spectral data the waveform was reconstructed.[8,9]

The recording time base was 60 ms and consisted of 2,048 digitized points. The lowest discernible frequency of the digital filter was 16.7 Hz (1/time base). The highest discernible frequency is given by the Nyquist equation:

Highest discernible frequency =
1/[time base/(number of points digitized/2)]

and was equal to 17,067 Hz. After filtering, the first 256 points (15 ms) were segmented and displayed for waveform analysis.

The latency of the IV/V peak and IV/V-Na1 amplitude was measured on all waveforms. Waveform identification was performed in accordance with previous reports.[6,10] The slow component of the auditory brain stem response was defined as Pϕ.[6,11] In many of the auditory brain stem responses studied in this report, the sharp IV and V peaks were absent and differentiation of these peaks from the slow component of the auditory brain stem response (Pϕ) was impossible. Na1 was defined as the major trough that followed the IV/V peak and occurred within the 15-ms time window. Na was defined as the lowest trough between Pϕ (slow component of the auditory brain stem response) and Pa. The P wave was defined as the positive peak that divided Na into the two troughs Na1 and Na2.[12]

The mean, standard deviation, and variance were calculated for all latencies and amplitudes. Statistical differences in mean latency and amplitude data were determined with a two-way analysis of variance (ANOVA). Analysis of the differences in latency and amplitude of all zero, standard, and unfiltered waveforms, regardless of the slope and cutoff frequency of the filter, was also performed by use of a two-way ANOVA with combined data groups. Statistical differences in variance data were determined with an F-max test.

RESULTS

Three of the 23 subjects had a large time-linked muscle potential. They were eliminated from the data analysis, since their responses were not primarily neurogenic but instead were neurogenic and myogenic. These subjects will have a response with a different phase and power spectral content than subjects with a primarily neurogenic response. Thus, the results of filtering will differ. The results of filtering waveforms with a large time-linked muscle potential are largely dependent upon the muscle potential's amplitude. Standard phase shift filtering can result in severe distortion of the waveform. Zero phase shift filtering may cause a deepening of the trough between wave IV/V and the potential, with the IV/V-Na1 amplitude obtaining a value of 2 to 3 μV.[13] For this reason these subjects were eliminated from data analysis. Subjects with a large amount of spontaneous brain activity or random muscle artifact were not eliminated from data analysis.

The results of zero and standard phase shift digital filtering on the auditory brain stem response from the other 20 subjects are shown in the Figure and Tables 1 through 8. As expected, phase shifting high pass filters, compared to the zero phase shift condition, resulted in a decrease in wave latency. Paired *t* test analyses showed all three filter comparisons (100 Hz, 36 dB/octave; 100 Hz, 12 dB/octave; and 150 Hz, 12 dB/octave) to be significant ($p < .003$, $p < .02$, and $p < .015$, respectively). Since over 20 analyses were undertaken, there is an increased probability of a significant result due to chance alone (type I errors). Thus, a two-way ANOVA of the individual filter groups was performed. This analysis revealed that only the standard phase shift 36-dB/octave filter produced a sig-

TABLE 1. WAVE IV/V LATENCY WITH ZERO AND STANDARD PHASE SHIFT HIGH PASS (100 HERTZ, 36 DECIBELS/OCTAVE) DIGITAL FILTERING

	Zero Phase Shift (ms)	*Standard Phase Shift (ms)*	*Significance*
Wave V latency	6.885	6.215	$p<.0001$*
Standard deviation	0.370	0.828	
Variance	0.137	0.685	$p<.01$, (ratio = 5.0)†

	Unfiltered (ms)	*Zero Phase Shift (ms)*	*Significance*
Wave V latency	6.780	6.885	NS ($p<.38$)*
Standard deviation	0.519	0.370	
Variance	0.270	0.137	NS (ratio = 1.97)†

	Unfiltered (ms)	*Standard Phase Shift (ms)*	*Significance*
Wave V latency	6.780	6.215	$p<.0001$*
Standard deviation	0.519	0.828	
Variance	0.270	0.685	$p<.05$ (ratio = 2.53)†

NS — not significant.

*Two-way analysis of variance.

†F-max test (variance ratio for $p<.05 = 2.46$; $p<.01 = 3.32$).

nificant latency reduction. (This reduction was also significant when compared to all other filter conditions.) This analysis assumed that all measurements are independent of each other. Because the variances in the data groups were not equal the ANOVA was a very conservative test, creating type II errors. Also, the ANOVA probably underestimated the true significance in the differences because all three measurements, if studied by themselves, had significant paired *t* tests. For this reason a two-way ANOVA with combined data groups was performed. When analyzed as a group, the filter conditions differed with a p value of less than .0001 (one chance in 10,000 that the results are due to chance alone). This significance was much higher than those found in any individual group and is a very strong argument that the latency reductions seen in the three phase shift filter conditions compared to the zero phase shift conditions are real and not due to chance. However, the changes were small for the 12-dB/octave filters (100 and 150 Hz), with wave IV/V having a similar mean latency and variance between the zero and standard phase shift conditions. This latency reduction was observed consistently in the majority of subjects studied. Zero phase shift filtering did not produce a significant shift in the IV/V wave latency compared to the unfiltered condition.

The response amplitude was larger with zero phase shift than standard phase shift filtering for all high pass conditions (Tables 2-4). A two-way ANOVA with combined data groups compared all zero to all standard phase shift conditions and found this difference was significant at the $p<.0001$ level. A two-way ANOVA found these differences to be significant at the $p<.0001$, $p<.0553$, and $p<.0466$ levels for the 100-Hz, 36-dB/octave; 100-Hz, 12-dB/octave; and 150-Hz, 12-dB/octave filters, respectively. Increasing filter slope from 12 dB/octave to 36 dB/octave resulted in an amplitude reduction for only the phase shift condition. Little effect was noted with increasing the filter slope for the zero phase shift condition (Table 5). Increasing the filter's cutoff frequency from 100 to 150 Hz resulted in a further amplitude reduction (14%) for the zero shift condition. Phase shifting the waveform only reduced the response by an additional 2% over the zero phase shift (150 Hz) condition (Table 6).

Phase shifting was responsible for the major decrease in amplitude with the 100-Hz, 36-dB/

TABLE 2. WAVE IV/V-NaI AMPLITUDE WITH ZERO AND STANDARD PHASE SHIFT HIGH PASS (100 HERTZ, 36 DECIBELS/OCTAVE) DIGITAL FILTERING

	Zero Phase Shift (μV)	*Standard Phase Shift (μV)*	*Significance*	*% Reduction*
Wave V amplitude	0.420	0.295	$p<.0001$*	30
Standard deviation	0.138	0.112		
Variance	0.019	0.031	NS (ratio = 1.63)†	

	Unfiltered (μV)	*Zero Phase Shift (μV)*	*Significance*	*% Reduction*
Wave V amplitude	0.499	0.420	$p<.0001$*	16
Standard deviation	0.156	0.138		
Variance	0.024	0.019	NS (ratio = 1.26)†	

	Unfiltered (μV)	*Standard Phase Shift (μV)*	*Significance*	*% Reduction*
Wave V amplitude	0.499	0.295	$p<.0001$*	41
Standard deviation	0.156	0.112		
Variance	0.024	0.031	NS (ratio = 1.29)†	

NS — not significant.

*Two-way analysis of variance.

†F-max test (variance ratio for $p<.05 = 2.46$; $p<.01 = 3.32$).

TABLE 3. WAVE IV/V-Nai AMPLITUDE WITH ZERO AND STANDARD PHASE SHIFT HIGH PASS (100 HERTZ, 12 DECIBELS/OCTAVE) DIGITAL FILTERING

	Zero Phase Shift (μV)	*Standard Phase Shift (μV)*	*Significance*	*% Reduction*
Wave V amplitude	0.407	0.368	p<.0553*	10
Standard deviation	0.127	0.111		
Variance	0.016	0.012	NS (ratio = 1.33)†	

	Unfiltered (μV)	*Zero Phase Shift (μV)*	*Significance*	*% Reduction*
Wave V amplitude	0.499	0.407	p<.0001*	18
Standard deviation	0.156	0.127		
Variance	0.024	0.016	NS (ratio = 1.5)†	

	Unfiltered (μV)	*Standard Phase Shift (μV)*	*Significance*	*% Reduction*
Wave V amplitude	0.499	0.368	p<.0001*	26
Standard deviation	0.156	0.111		
Variance	0.024	0.012	NS (ratio = 2.0)†	

NS — not significant.

*Two-way analysis of variance.

†F-max test (variance ratio for p<.05 = 2.46; p<.01 = 3.32).

octave standard phase shift high pass filter, as response energy elimination was the major factor in the decrease of amplitude with the 150-Hz standard phase shift high pass filter.

Steep filters (36 dB/octave) produced severe response distortion (see Figure) that made waveform identification difficult. This distortion often caused the slow component of the auditory brain stem response to align itself with waves III and I (Table 8). In several cases, wave III had a latency greater than 5 ms and was the major response peak, with wave IV/V being smaller or absent (see Figure, waveforms 3S and 7S).

The amplitude reduction for the 100-Hz, 36-dB/octave zero phase shift filter was less than that for the 150-Hz, 12-dB/octave standard phase shift filter (Table 7). The larger waveforms in the zero phase shift condition were caused by a greater preservation of response energy (as shown by the two zero phase shift comparisons in the Figure) and by the lack of phase shift distortions.

The only significantly different latency variance was for the 36-dB/octave standard filter. This variance differed from all other filter settings. Analysis of the individual data revealed that six of the 20 data points would have to be discarded to eliminate this significance. It is unlikely that this finding is due to chance since over one quarter of the data points were responsible for this variance. The Figure also demonstrates that this variance was due

TABLE 4. WAVE IV/V-Nai AMPLITUDE WITH ZERO AND STANDARD PHASE SHIFT HIGH PASS (150 HERTZ, 12 DECIBELS/OCTAVE) DIGITAL FILTERING

	Zero Phase Shift (μV)	*Standard Phase Shift (μV)*	*Significance*	*% Reduction*
Wave V amplitude	0.351	0.310	p<.0466*	12
Standard deviation	0.110	0.103		
Variance	0.012	0.011	NS (ratio = 1.09)†	

	Unfiltered (μV)	*Zero Phase Shift (μV)*	*Significance*	*% Reduction*
Wave V amplitude	0.499	0.351	p<.0001*	30
Standard deviation	0.156	0.110		
Variance	0.024	0.012	NS (ratio = 2.00)†	

	Unfiltered (μV)	*Standard Phase Shift (μV)*	*Significance*	*% Reduction*
Wave V amplitude	0.499	0.031	p<.001*	38
Standard deviation	0.156	0.103		
Variance	0.024	0.011	NS (ratio = 2.18)†	

NS — not significant.

*Two-way analysis of variance.

†F-max test (variance ratio for p<.05 = 2.46; p<.01 = 3.32).

TABLE 5. WAVE IV/V-Na1 AMPLITUDE COMPARISON BETWEEN HIGH PASS FILTERS OF 100 HERTZ (SLOPES OF 36 DECIBELS/OCTAVE AND 12 DECIBELS/OCTAVE)

	Zero Phase Shift 36 dB/Octave (μV)	*Zero Phase Shift 12 dB/Octave (μV)*	*Significance*	*% Reduction*
Wave V amplitude	0.420	0.407	p<.5145*	3
Standard deviation	0.138	0.127		
Variance	0.019	0.016	NS (ratio = 1.19)†	

	Zero Phase Shift 36 dB/Octave (μV)	*Standard Phase Shift 12 dB/Octave (μV)*	*Significance*	*% Reduction*
Wave V amplitude	0.420	0.368	p<.0108*	12
Standard deviation	0.138	0.111		
Variance	0.019	0.012	NS (ratio = 1.58)†	

NS — not significant.

*Two-way analysis of variance.

†F-max test (variance ratio for p<.05 = 2.46; p<.01 = 3.32).

to the severe distortion produced by the filter and not by chance occurrence.

DISCUSSION

The digital filtering techniques used in this study differed from those of previous reports in that the filter's resolution was 16 Hz. Other authors have studied digital models of analog filters with much lower resolutions (from 83 to 111 Hz—estimated from the reciprocal of the time bases given in the Methods section or shown in the Figure).[14-17] A filter resolution of 16.7 Hz is several octaves below the cutoff frequencies of the studied filters, thus increasing the validity of the data reported in this study.

High pass analog filters (cutoff frequencies of 100 and 150 Hz) phase-shift and distort the auditory evoked potentials. It is the phase shifting of the 100-Hz energy in relation to the higher response energy (500- and 1,000-Hz spectral peaks) that causes the primary distortion. The high frequency component of the auditory brain stem response (500-Hz spectral peak) is more than an octave above the filter's cutoff frequency and is not phase shifted as much as the lower frequency energy. Also, the smaller wave period resulted in a much smaller latency shift per degree of phase shift.

Using steep analog filters to eliminate spontaneous brain activity and random muscle artifact is not desirable because of the severe response distortions caused by phase shifting. By comparing the data of the 100-Hz, 36-dB/octave high pass filter in Tables 1 and 2 the following conclusions can be reached. First, phase shifting caused a significant amplitude reduction in the recorded response in the majority of patients (16/20). With zero phase shift filtering the IV/V-Na1 amplitude was reduced by only 16% (p<.0001) as the phase-shifted response was reduced 41% (p<.0001) as compared to the unfiltered control. The difference in these two values was due to the phase characteristics of the filter and was significant at the p<.0001 level. The amplitude reduction in the phase-shifted waveform is caused by a nonalignment of the response energy peaks. This results in higher energy peaks being shifted into the troughs of lower energy peaks.[10]

Compared to previous studies,[14-16] the observed amplitude reduction with use of a zero phase shift

TABLE 6. WAVE IV/V-Na1 AMPLITUDE COMPARISON BETWEEN HIGH PASS FILTERS WITH SLOPE OF 12 DECIBELS/OCTAVE (CUTOFF FREQUENCIES OF 100 HERTZ AND 150 HERTZ)

	Zero Phase Shift 100 Hz (μV)	*Zero Phase Shift 150 Hz (μV)*	*Significance*	*% Reduction*
Wave V amplitude	0.407	0.351	p<.0057*	14
Standard deviation	0.127	0.110		
Variance	0.016	0.012	NS (ratio = 1.33)†	

	Zero Phase Shift 100 Hz (μV)	*Standard Phase Shift 150 Hz (μV)*	*Significance*	*% Reduction*
Wave V amplitude	0.368	0.310	p<.0001*	16
Standard deviation	0.111	0.103		
Variance	0.012	0.011	NS (ratio = 1.09)†	

NS — not significant.

*Two-way analysis of variance.

†F-max test (variance ratio for p<.05 = 2.46; p<.01 = 3.32).

TABLE 7. WAVE IV/V-Na1 AMPLITUDE COMPARISON BETWEEN HIGH PASS FILTERS OF 100 HERTZ (36 DECIBELS/OCTAVE) AND 150 HERTZ (12 DECIBELS/OCTAVE)

	Zero Phase Shift 100 Hz, 36 dB/Octave (μV)	*Zero Phase Shift 150 Hz, 12 dB/Octave (μV)*	*Significance*	*% Reduction*
Wave V amplitude	0.420	0.351	$p<.0007$*	16
Standard deviation	0.138	0.110		
Variance	0.019	0.012	NS (ratio = 1.58)†	
	Zero Phase Shift 100 Hz, 36 dB/Octave (μV)	*Standard Phase Shift 150 Hz, 12 dB/Octave (μV)*	*Significance*	*% Reduction*
Wave V amplitude	0.420	0.310	$p<.0001$*	26
Standard deviation	0.138	0.103		
Variance	0.019	0.011	NS (ratio = 1.73)†	

NS — not significant.

*Two-way analysis of variance.

†F-max test (variance ratio for $p<.05=2.46$; $p<.01=3.32$).

filter was slightly larger. This discrepancy is probably due to the higher resolution and steeper slope (36 dB/octave) of our digital filter, along with our use of a lower stimulus intensity. The lower stimulus intensity will not elicit well-defined high frequency components of the auditory brain stem response (the sharp peaks of waves IV and V) and can cause an increase in the latency of the Na1 trough out of proportion to the increase in latency of wave IV/V. This latter effect is caused by the P wave being positioned on the down shoulder of the slow component (Pϕ) of the auditory brain stem response at high stimulus intensities and the up shoulder of wave Pa at low intensities.[6] The slow component of the auditory brain stem response widens and the frequency of the response is lowered. The 15-ms time base used in our study allowed the recording of the prolonged Na1, which can have a latency greater than 10 ms at low stimulus intensities.[13] The widening of the slow component along with the mitigation of high frequency components will produce a lowering of the mean spectral content of the response.

Besides significant amplitude effects, steep analog filters (36 dB/octave) can cause a large decrease in wave latency (0.67 ms; $p<.0001$) and an increase in wave latency variability (250%; $p<.05$) (Table 1). This increase in variability is caused by two factors. First, phase shifting can severely distort the response, making wave identification difficult (see Figure). This effect can cause the slow component of the auditory brain stem response to align itself with wave III. The sharp peaks of wave IV/V are often absent or markedly diminished in low intensity recordings. In all unfiltered and in 19 of 20 zero phase shift waveforms the peak of the auditory brain stem response was at wave IV/V. However, in the standard phase shift condition the slow component of the auditory brain stem response decreased in latency out of proportion to waves I through V. The peak of the auditory brain stem response varied between waves I, III, and IV/V (Table 8). Since a low intensity stimulus was used, all peaks were delayed and ill defined. Thus, confusion was created regarding waveform identification. This confusion led to the misreading of several of the waveforms. Wave III was often delayed past 5.0 ms and phase shifting can cause it to be the largest waveform of the response. Different observers may disagree on the location of wave IV/V (Pϕ) in the analog-shifted waveforms. One cannot eliminate this confusion and thus it represents a confounding factor in latency determination of analog-filtered (phase-shifted) responses. This is especially evident in subjects 3S and 7S (see Figure). Second, because all auditory evoked potentials have a different spectral composition the degree of shift will differ and thus further the increase in variability of the latency measurement. Filtering the amplitude spectrum will cause a variability in wave latency for both standard and zero phase filters. However, the added change in the phase spectrum of waveforms modified by standard phase shift or analog filters will cause an increase in this variability. A small latency variability is very desirable in neurodiagnosis, in which small normative standard deviations and accurate latency

TABLE 8. LOCATION OF HIGHEST PEAK IN FIRST 8 MILLISECONDS OF RESPONSE

Filter Characteristics	*Wave I*	*Wave III*	*Wave IV/V*
Unfiltered	0	0	20
36 dB/octave high pass 100 Hz			
Zero phase shift	0	1	19
Standard phase shift	5	10	5
12 dB/octave high pass 100 Hz			
Zero phase shift	0	1	19
Standard phase shift	0	5	15
12 dB/octave high pass 150 Hz			
Zero phase shift	0	0	20
Standard phase shift	0	6	14

Peak in standard recording was compared to zero phase-shifted recording in order to identify highest peak location (Pϕ).

measurements increase the reliability of the test.

The waveform distortions (amplitude reductions) from phase shifting 12-dB/octave filters were not as great as those found with 100-Hz, 36-dB/octave phase shifting filters ($p < .0003$) (Tables 2 and 3). Also, the 12-dB/octave standard phase shift high pass filters of 100 and 150 Hz had similar wave latency variances and mean amplitude differences between the zero and standard phase shift conditions. Thus, the clinical use of shallowly sloping filters to avoid severe phase shift distortions appears to be valid.

However, one should be careful when using a high pass cutoff frequency to compensate for the shallower filter slope. The amplitude reduction of the auditory brain stem response filtered with a 150-Hz, 12-dB/octave standard phase shift high pass filter approximated that obtained with a 100-Hz, 36-dB/octave standard phase shift high pass filter (38% versus 41%, respectively) (Tables 2 and 4). At a cutoff frequency of 100 Hz, the 36-dB/octave standard phase shift filter produced a much smaller (30%) wave IV/V-Na1 amplitude than the zero phase shift condition (Table 2), as a cutoff frequency of 150 Hz produced little difference (12%) between the two amplitudes (Table 4). It can be theorized that the amplitude reduction with the 150-Hz, 12-dB/octave standard phase shift filter is primarily due to the elimination of response energy and that seen in the 100-Hz, 36-dB/octave standard phase shift filter is primarily due to phase shifting (Tables 5 and 6).

This is further confirmed by the finding that a shallow (12-dB/octave) zero phase shift filter with a cutoff frequency of 150 Hz eliminated significant response energy with a resultant 30% decrease in IV/V-Na1 amplitude compared to the unfiltered condition (Table 4). This reduction is more than the 16% to 18% obtained with the 100-Hz, 36-dB/octave and 12-dB/octave zero phase shift filter (Tables 2 and 3). It is apparent that high pass analog filtering at 150 Hz not only phase-shifts the response but also results in significant elimination of response energy.

Varying the slope of the zero phase shift filter at 100 Hz did not have a marked effect on the IV/V-Na1 amplitude (Table 5). This finding can be attributed to the filtering of the 100-Hz spectral peak of the auditory brain stem response. A shallowly sloping filter will eliminate more energy above the cutoff frequency than a steeply sloping filter, as a steeply sloping filter will eliminate more energy below the filter's cutoff frequency than a shallowly sloping filter. When the filter's cutoff frequency approximates the response energy, the relative effects on amplitude cannot be predicted.

The amplitude reduction of a 12-dB/octave standard phase shift high pass filter at 150 Hz was 26% more than the reduction recorded with a 36-dB/octave zero phase shift filter at 100 Hz (Table 7). This finding clearly demonstrates the advantages of zero phase shift digital filters in preservation of response amplitude. Unfortunately, digital filtering cannot be performed during signal averaging with commercially available evoked response units. It is hoped that with the falling price of computer components and the production of faster processors that this hardware will soon be available.

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COMPUTER-AIDED THREE-DIMENSIONAL RECONSTRUCTION: A METHOD OF MEASURING TEMPORAL BONE STRUCTURES INCLUDING THE LENGTH OF THE COCHLEA

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To visualize the three-dimensional (3-D) shapes of structures in the temporal bone from histologic sections, we developed computer software to create computer-generated 3-D images from two-dimensional (2-D) materials. Using those images, we have been able to measure these structures in three dimensions. This means that quantitative 3-D measurements can be performed regardless of the angle at which the specimen was cut. This report describes this method for 3-D reconstruction and measurement and discusses its implications. For instance, in addition to showing the 3-D shape of the cochlea from different angles, we have been able to measure the length of the cochlea and have shown that measurements made by use of the conventional 2-D graphic reconstruction method can be distorted considerably by variations in cutting angle of the specimen.

KEY WORDS — length of cochlea, morphometry, temporal bone, three-dimensional reconstruction.

INTRODUCTION

Traditional methods for studying the temporal bone histologically involve cutting the specimen into thin serial sections and viewing them under a light microscope. Cutting the temporal bone into thin sections is necessary for staining the specimen and evaluating the histology under high-power magnification. On the other hand, these procedures make it difficult to recognize the three-dimensional (3-D) shape of structures in the temporal bone. This is a significant disadvantage of traditional histologic methods, because the spatial orientation of inner ear structures, in particular, is related to their function. An additional problem with traditional temporal bone histology methods is that the dimensions of structures in two-dimensional (2-D) histologic sections often vary with differences in the cutting angle of the specimen. Hence it is almost impossible to reconstruct the 3-D shape of the temporal bone by looking at 2-D sections.

To solve these problems, we developed a method for generating 3-D images of temporal bone sections by use of a personal computer. Further, we have developed ways to use the 3-D image to measure structures in three dimensions, regardless of the angle at which the specimen was cut. This means that for the first time, quantitative morphometric study of the temporal bone is possible.

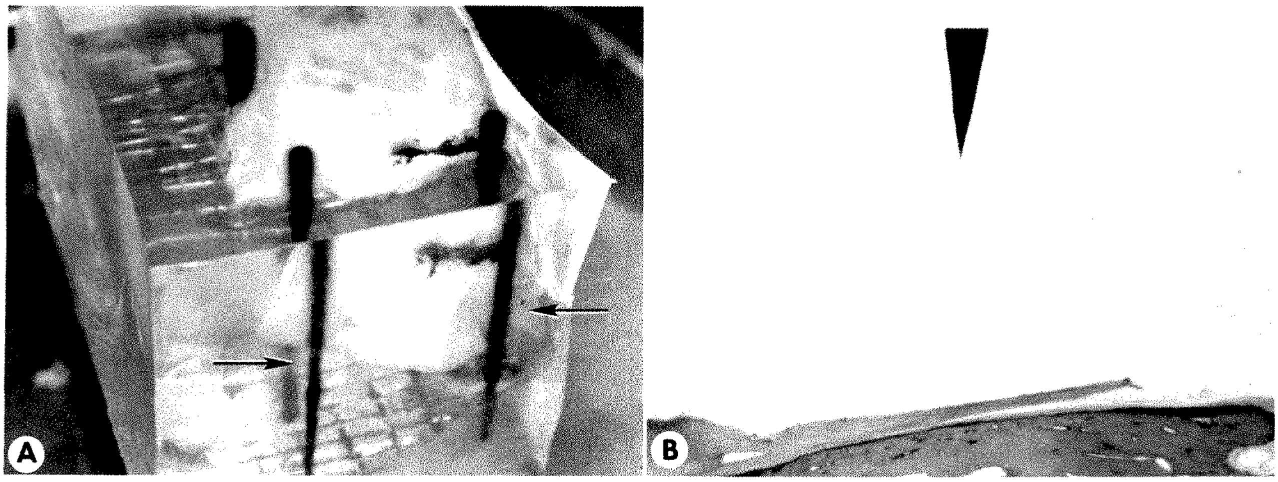

Fig 1. Preparation of temporal bone specimen. A) Celloidin block containing temporal bone and reference lines (arrows). Reference lines are made parallel to each other and perpendicular to cutting surface adjacent to temporal bone specimen. B) Reference point (arrowhead) in finished specimen. Black Magic Marker ink persists in hole in celloidin.

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REPRINTS — Isamu Sando, MD, DMS, Division of Otopathology, Eye and Ear Institute, 203 Lothrop St, Pittsburgh, PA 15213.

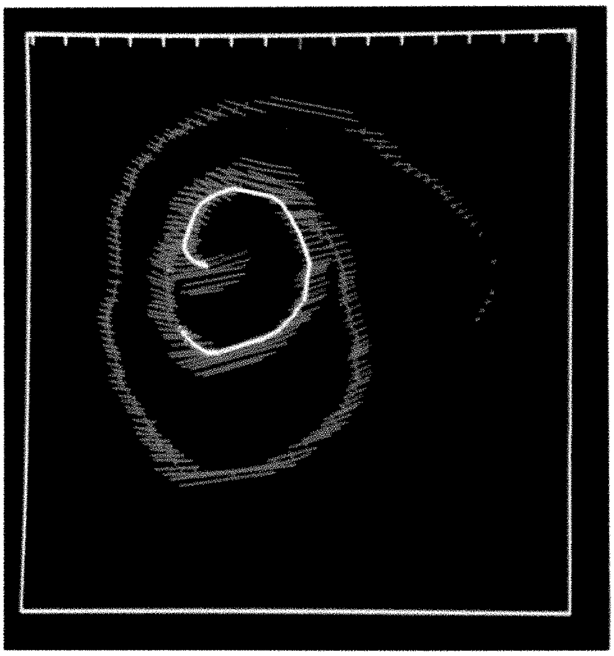

Fig 2. Measurement of cochlear length. Green lines indicate membranous spiral lamina on which pillar cells are present in histologic sections. Measurement was made from apical end, along white line that connected union of pillar cells in each section. Red lines indicate structures in particular section (plane) on which cursor could be moved. Length of white line to this point was 7.51 mm.

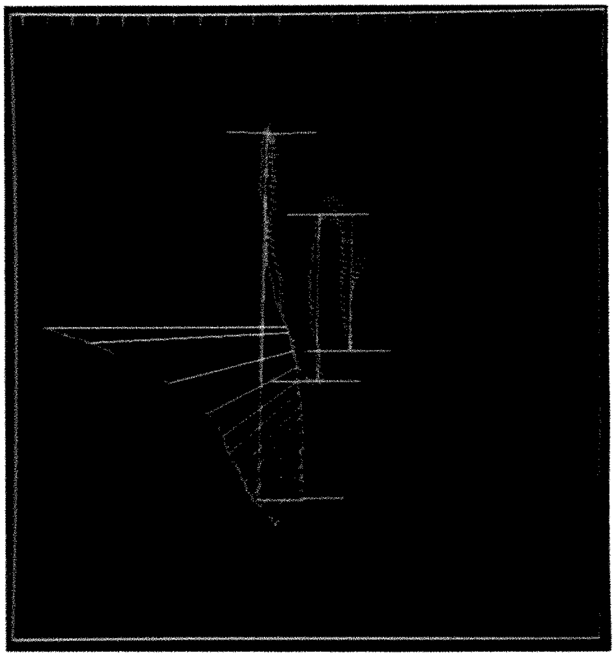

Fig 3. Measurement of cochlear turn diameters and hook portion on new 2-D reconstruction after correcting position of cochlea for oblique cutting angle. Vertical (pink) lines indicate diameters for each turn. Horizontal lines (white), which are in planes parallel to plane of midmodiolus, indicate distance between union of pillar cells in anterior basal turn and those in hook portion. From these distances and their depths from top of basal turn, it was possible to draw new 2-D graphic reconstruction of hook portion of cochlea and cochlear turns.

This paper reports our method for 3-D reconstruction of the temporal bone and shows its usefulness with an example: measurement of the true length of the cochlea. This is the first report of a difference in length of the cochlea as determined by 3-D reconstruction and traditional 2-D graphic reconstruction.

METHOD AND MATERIALS

System and Software. The equipment consists of a personal computer (NEC 98-XA), a high-resolution (1,120 x 750 pixels) 14-inch color monitor, a digitizer (Hitachi HDG-1111) with resolution set at 0.1 mm, a plotter (Roland DSY-980), and a printer. The task-specific software was developed by use of a BASIC interpreter and its compiler, which enables us to rotate, enlarge or reduce, and move the 3-D figures freely. Furthermore, images appearing on the color monitor can be used to identify any point in three dimensions by use of the digitizer tablet. This is done by indicating the x and y coordinates by movement of the tablet cursor, while the z coordinate is entered by moving from one x-y plane to the next and is displayed in a different color. Such coordinate registration enables us to do morphometric calculations on the monitor screen. With this method, we can measure the following parameters in three dimensions: length, angle, area (surface), and volume. The angle between two planes, as well as between two lines in space, also can be measured.

Procedure and Materials. Temporal bone specimens are removed at autopsy, fixed, decalcified, and embedded in celloidin. Before cutting the specimen, we put reference holes into the hardened celloidin block at three points adjacent to the temporal bone specimen. These reference lines are made parallel to each other and as close to perpendicular to the cutting surface as possible (Fig 1A). To make the reference holes, we coated the surface of a rounded needle (25-gauge) with Magic Marker ink and then inserted the needle into the celloidin block, guided by the holes in a wooden block placed over the specimen. The insertion of the needle coated with marker ink was repeated several times to ensure good staining of the hole.

The specimen was cut horizontally at 20 to 30 μm, according to the condition of the specimen. The black Magic Marker ink remains around the edges of the hole in the celloidin, even after staining, so that the reference marks can be identified in the finished slides (Fig 1B). Next, each histology section was projected onto paper at 16.5x magnification, and the structures to be studied and reference marks were traced onto the paper. Then, the papers were stacked in the order in which the histologic sections were cut, and with all of the reference marks aligned. Finally, we entered the data into the computer by tracing each structure on each of the

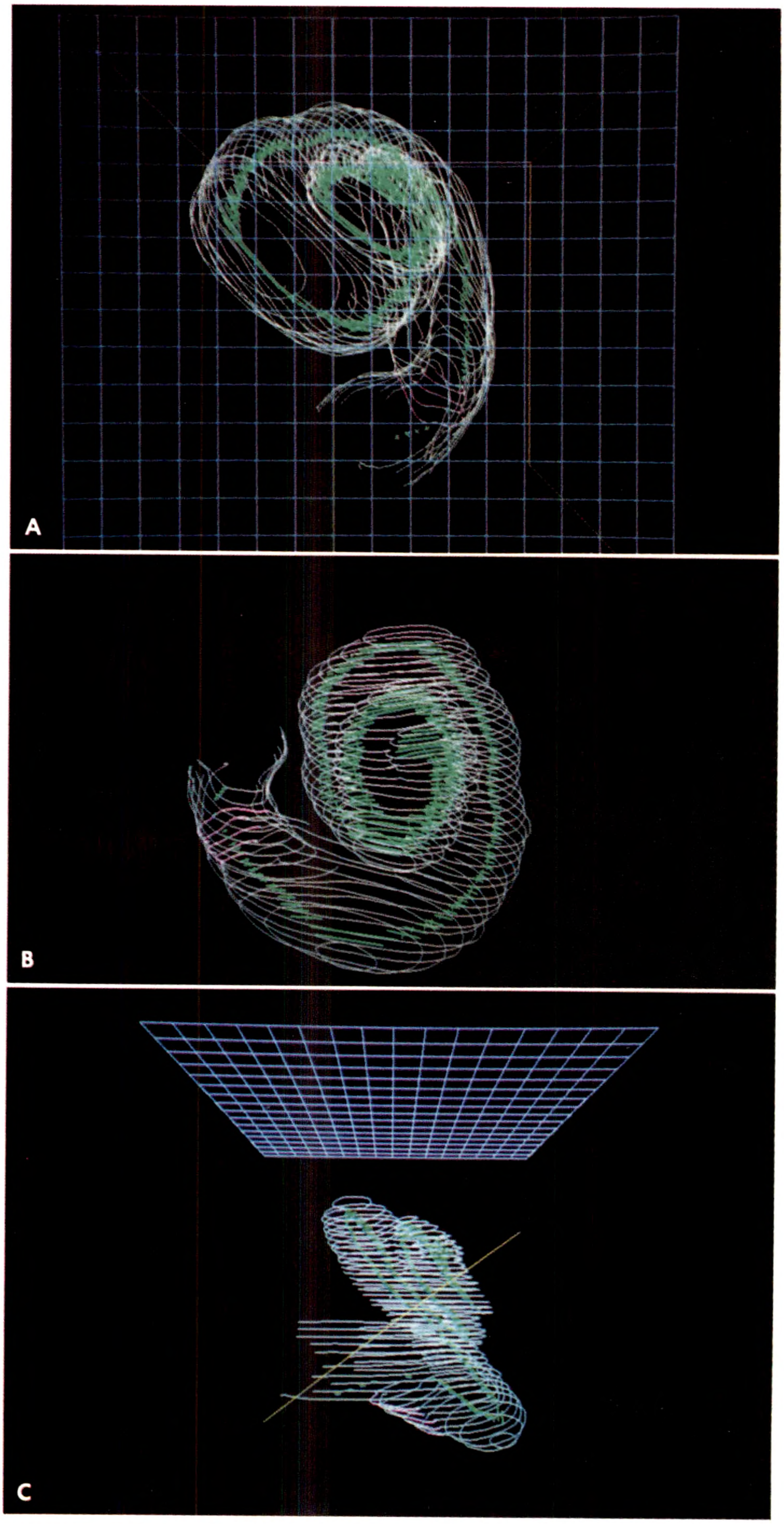

Fig 4. Cochlea. A) Seen from plane of cutting. Axis is upward. B) Frontal aspect (anterolateral view). C) Side aspect (posterolateral view). In this view, it is clear that cochlea was not cut parallel to axis of midmodiolus. Blue grid represents cutting plane.

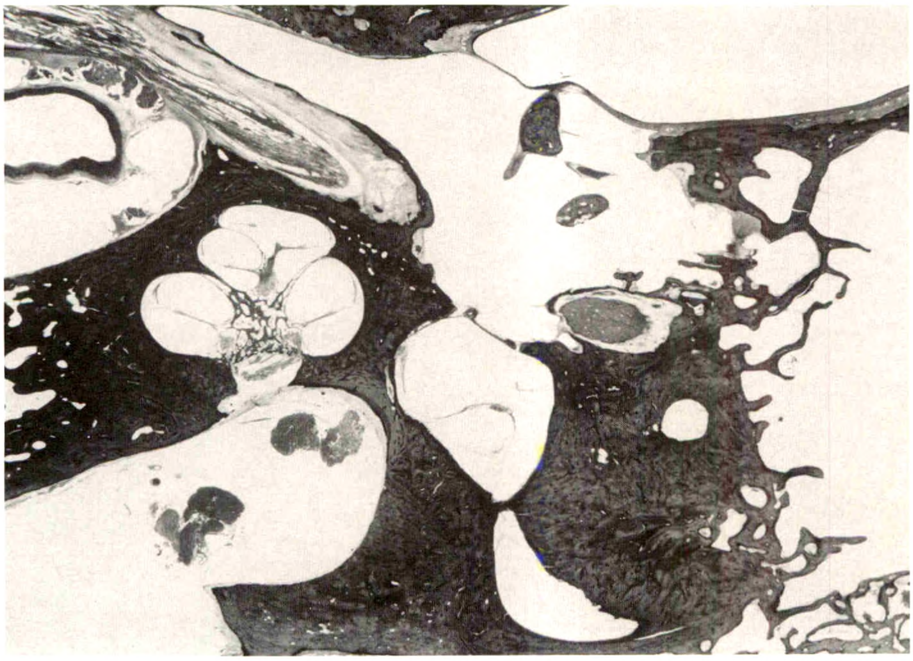

Fig 5. Midmodiolar histologic section of this temporal bone. Cochlea seems to have been cut parallel to axis of midmodiolus.

papers on the digitizer, using a different color for each structure. Also, data on the thickness of the sections were entered as z coordinates. Data entry simultaneously generated 3-D images of the structures on the monitor as a wire frame model displayed in perspective.

Although it seemed to be difficult to measure 3-D structures using 2-D devices, such as the x-y digitizer tablet or monitor screen, this was made simpler by the fact that our original data did not include a continuous line along the z-axis. Thus, as long as we could move the cursor from a point on one x-y plane to the corresponding point on the next x-y plane, it was possible to identify three-dimensionally (ie, on the z-axis) any point we had entered by using the x-y tablet. This is facilitated by the fact that structures on any particular plane in which the x-y cursor is moving can be shown in a certain color on the monitor screen.

We also could use another coordinate system for cursor movement. The primary coordinates were defined by the angle at which the specimen was cut. However, to view the specimen from another anatomic view, and to adjust the cursor movement for it, we could define a second set of coordinates. This ability to define a second set of coordinates is also very useful in that it permits us to draw auxiliary lines for the complement of the 2-D devices.

In this particular example for the application of this system, we measured the length of the cochlea spiral at the point at which the pillar cells joined, using the temporal bone from a 76-year-old man who had died from myocardial infarction. This was calculated by entering the point of union of the pillar cells in each section sequentially (Fig 2). In this study, we also calculated the length of the cochlea from a 2-D graphic reconstruction of the cochlea, using the method developed by Guild[1] as revised by Schuknecht[2]; this method has long been used by temporal bone researchers to measure cochlear length and shape.

Because we know that the angle at which the specimen was cut was oblique, we simulated cutting the specimen parallel to the midmodiolus. We

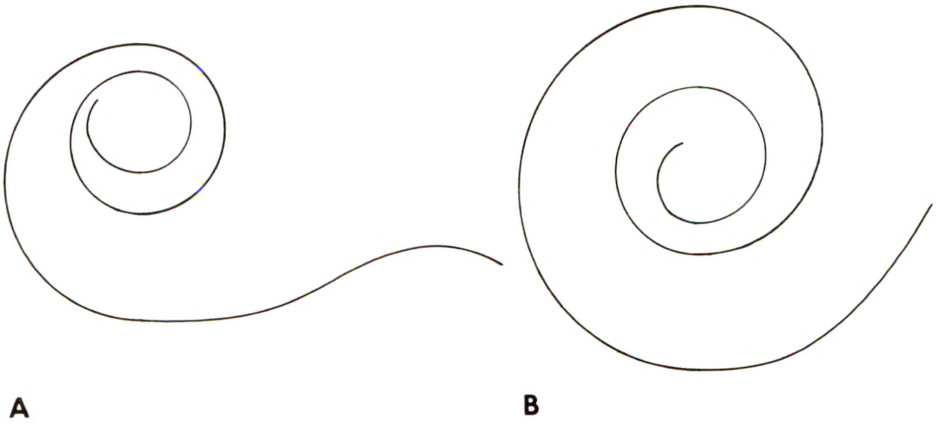

Fig 6. Comparison of 2-D graphic reconstructions of cochlea. Lengths and shapes are quite different. A) Original position (30.8 mm). B) Corrected position (36.3 mm).

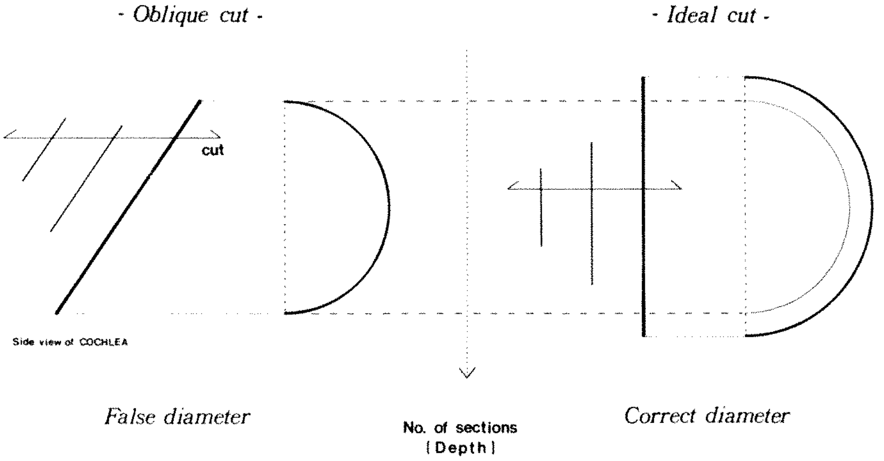

Fig 7. Schema of 2-D graphic reconstruction of cochlear turns. If cochlea is cut obliquely, apparent (false) diameters of turn are smaller than actual (correct) diameters.

then performed a new series of measurements in 3-D on this computer simulation of the cochlea to draw an angle-corrected 2-D graphic reconstruction for comparison with the original one (Fig 3).

RESULTS

The shape of the cochlea as seen from the original angle at which the sections were cut is shown in Fig 4A. The green lines represent the union of pillar cells. From this original image, the computer generated the image of the cochlea from the frontal aspect (anterolateral view; Fig 4B) and from the side aspect (posterolateral view; Fig 4C). From the side view, it is clear that this cochlea was not cut parallel to the axis of the midmodiolus. The angle of cutting was in fact found to be 35° from the midmodiolus of the cochlea, even though in the histologic sections the modiolus appeared to have been cut parallel to its axis (Fig 5).

The length of the cochlea in 3-D was 36.4 mm in this particular case. On the other hand, the value measured by the 2-D cochlear graphic reconstruction method (Fig 6A) was 30.8 mm for the same case. The difference was 5.6 mm (15.4%), which is considerable. The most probable source of the difference was the magnitude of the angle between the plane of cutting and the axis of the midmodiolus of the cochlea. Therefore, we performed a computer simulation of cutting the cochlea parallel to the axis of the midmodiolus. When the oblique cutting angle (35°) was compensated, the 2-D length of the cochlea was 36.3 mm (Fig 6B), very close to the 3-D value (36.4 mm).

In addition to these morphometric differences, the shape of the corrected 2-D graphic reconstructions was quite different from that of the original 2-D graphic reconstruction, especially in the hook portion (Fig 6). In the computer-aided corrected 2-D graphic reconstruction, the downward curvature in the hook portion had disappeared, and the 2½ cochlear turns seemed to be more concentric and 32% larger (28.0 mm versus 21.2 mm); further, the length of the hook from the lowest part of the basal turn, corrected in the 2-D model (6.0 mm), is 1.5 mm shorter than in the original 2-D reconstruction (7.5 mm).

DISCUSSION

The advantages of a 3-D reconstruction technique, using a computer to study temporal bone histology, include

1. The ability to visualize the real 3-D shapes of structures in the temporal bone.
2. The ability to appreciate the 3-D relationship between structures, viewed from any direction and under any magnification by easy manipulation. Because the reconstructions are transparent, structures behind the plane being viewed can be seen, and it is easier to realize many relationships that cannot be seen readily in the intact specimen.
3. The ability to measure the structures in 3-D, regardless of the cutting angle. This enables us to do quantitative morphometry using temporal bone serial sections.

If we ignore the cutting angle effect on shape and morphometry, distorted and incorrect information will be derived from the study of temporal bone structures. To determine the original cutting angle, currently it is most practical to use a computer 3-D reconstruction method.

To demonstrate and explain the effect of the cutting angle problem in 2-D evaluation of temporal bones, Fig 7 shows the difference in cochlear length obtained by measuring it on the 2-D reconstruction of the cochlea as actually cut and by measuring it on the 2-D reconstruction of the cochlea as if it had been cut ideally (the angle-corrected 2-D reconstruction). If the cochlea is cut obliquely, the apparent diameters of the turns measured on the 2-D reconstruction are smaller than on the ideal reconstruction, because in the 2-D graphic method, which assumes that the specimen was cut parallel to the axis of the midmodiolus, the diameter is deter-

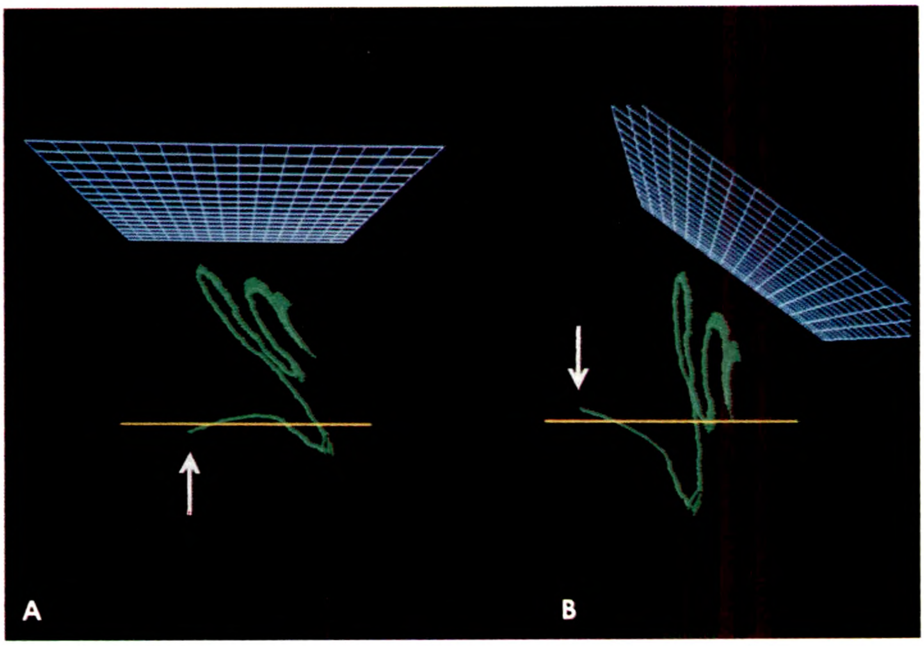

Fig 8. Contour of hook portion as seen from different cutting angles. A) In original position, end portion of hook is downward (arrow). B) In correct position, end is upward (arrow).

mined simply by adding the thickness of the sections. Thus, when a specimen is cut obliquely, as in our case, there is considerable error in comparison to the ideally cut specimen; in our case it was 15% (30.8 mm versus 36.3 mm) of the true total length of the cochlea. From our calculations on angle-corrected 2-D reconstruction, we knew that when the cutting angle is corrected for, the length of the cochlea in 2-D becomes quite similar to the 3-D value. Thus, we could confirm that the major source of error in measuring the length of the cochlea in 2-D graphic reconstruction was the cutting angle. So we realized that the angle of cutting always should be taken into consideration when performing measurements on temporal bone sections.

To appreciate the difference in the shape of the hook portion between the original and the corrected 2-D graphic reconstruction (Fig 6), the side (posterolateral) view of each in 3-D is presented in Fig 8. The end portion of the hook in the original reconstruction points downward, but in the corrected one it points upward. These are reflected in the shape of the hook portion in 2-D graphic reconstructions; thus, in truly midmodiolar sections, a downward curvature of the hook would not be seen.

The kind of cutting angle deviation seen here is not particular to this specimen, but seems to be common. This is because most "horizontal" temporal bone sections are cut parallel to the middle cranial fossa surface, which is not the horizontal plane anatomically, but tilts downward from it anterolaterally. In vertical sections (Fig 9), it is clear that the axis of the midmodiolus is never parallel to the middle cranial fossa surface, but rather that they form a considerable angle.

Ideally, before we undertake 2-D graphic reconstruction we should know if a specimen was cut exactly along the midmodiolar axis. However, this cannot be judged by looking at representative sections of the modiolus, because the modiolus itself is too thick to permit accurate judgment as to whether it was cut exactly along its axis (Fig 5). To make matters worse, if the modiolus was cut obliquely, there is no way to obtain the exact cochlear length except by using 3-D computer reconstruction.

The 3-D computer graphic reconstruction method requires many meticulous procedures. It is essential that the reference holes be made in the celloidin block before cutting. We put the reference holes parallel to each other and perpendicular to the cutting surface, in Magic Marker ink. Even though this is the method that worked best of the several that were tried, the marks are sometimes difficult to see because of the fading of the ink during the staining process. For the purpose of making the reference marks, drilling the bone might be better. The closer the reference marks are to the structures we want to reconstruct in the temporal bone, the easier and the more accurate the reconstruction becomes. However, it is not practical to make these marks too close, because there is a possibility of damaging other important structures, and because we usually do not make temporal bone sections specifically for 3-D reconstruction but for general histologic study.

Beyond this matter of reference marks, a fundamental requirement for any histologic study is that the sections have minimal artifacts, such as tearing, folding, waving, shrinkage, variations in thickness, and changes in cutting angle. Some artifacts are inevitable, as long as celloidin blocks are cut on conventional microtomes, but most can be avoided by careful manipulation of the specimen. If good tem-

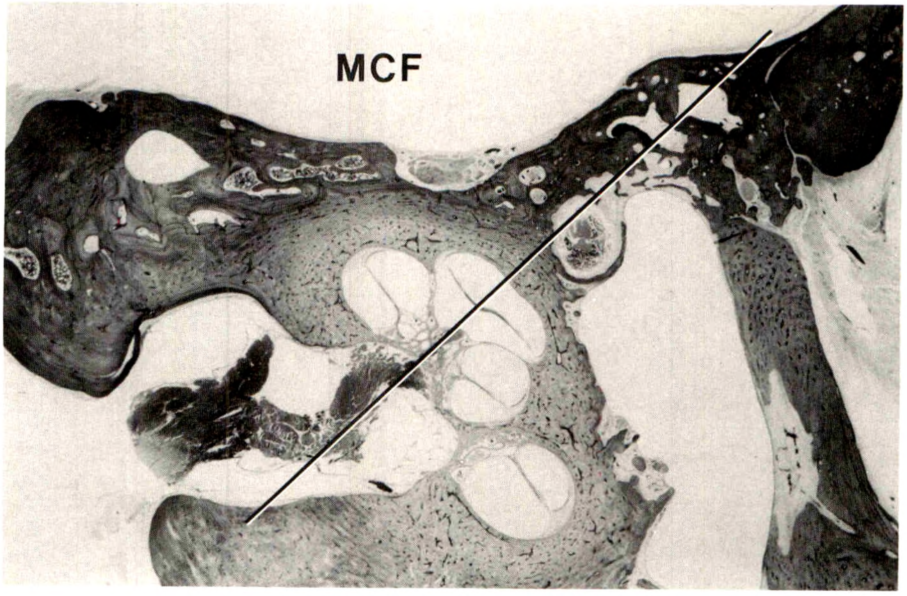

Fig 9. Vertical section through temporal bone. Axis of midmodiolus (black line) is tilted considerably upward toward middle cranial fossa (MCF).

poral bone sections can be obtained consistently, the computer reconstructions and measurements become much easier and more accurate. But much work must still be done manually, because the capacity of the computer to recognize patterns is far from ideal at present. Despite these drawbacks, we believe 3-D morphometry is the only technique by which the temporal bone may be studied quantitatively.

There were fewer reports in the literature on the length of the human cochlea than we had expected. Two types of methods of measuring this length have been described: indirect methods, such as the 2-D cochlear graphic reconstruction method, and direct methods, such as measurement of the segments of the cochlear turns after surface preparation of the specimen. Guild,[1] Hardy,[3] and Schuknecht[2] have described indirect methods and discussed the possible errors of these methods, but never referred to the cutting angle problems. If a specimen had been cut exactly through the midmodiolus, the error would be only a small percentage, but in practice there is much variation in cutting angle, which leads to much greater error.

Methods for direct measurement of cochlear length were described first by Retzius,[4] who used a micrometer, and later by Bredberg[5] and Ulehlova et al,[6] who measured the human cochlea using surface preparation techniques. Although this type of technique seems to be more reliable than indirect measurement, there is still the possibility of error due to destruction or loss of part of the organ of Corti during the segmentation of the cochlear turns by scissors. Besides, fragmentation of the cochlea means that the structure can never again be viewed in its original shape; this technique cannot be generalized as well as our computer method.

A new technique for generating 3-D images by computed tomography has been developing recently. But the application of this method to temporal bone study is limited, because the resolution obtainable by the most advanced CT methods (0.5 mm) is still too coarse to make precise definition of inner ear structures possible. For instance, the diameter of the semicircular canals or the short diameter of the stapes footplate would have to be recognized from only two or three tomographic slices. Further, discrimination of soft tissue structures, such as the membranous labyrinth, is difficult on CT because of the small differences in radiodensity between them and the surrounding structures. Thus, histopathologic study of the temporal bone is indispensable to precise morphometry of its structures. The method we describe in this report seems to be the best available at present.

In conclusion, although this 3-D reconstruction method requires a tremendous amount of time and many meticulous manual steps, we believe that it is a practical way to study the temporal bone morphometrically, because no other morphometric method is as accurate as the one we discuss. For example, the length of the cochlea was found to be 36.3 mm by computer-aided measurement, although it had been calculated to be 30.8 mm when measurements were made by the 2-D graphic reconstruction technique. It would be a serious problem if, whenever the function of the cochlea (hearing) is studied, the cochlea is assumed to be about 15% shorter than it actually is. We believe that the superiority of this method in providing accurate information about the basic anatomy of the temporal bone justifies the time and effort spent using this method. This technique also can be applied to the study of other structures of the temporal bone. Thus, this method opens up many possibilities for obtaining further valuable anatomic and pathologic information about the function of the ear.

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INDUCTION OF ANTIGEN-SPECIFIC IGA-FORMING CELLS IN THE UPPER RESPIRATORY MUCOSA

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We investigated the actual processes for activating respiratory mucosal immunity. Hartley guinea pigs were immunized with particulate antigen of dinitrophenylated ovalbumin into the duodenum (group A), trachea (group B), and nasal cavity (group C) 1 week after systemic priming with the soluble antigen. The control animals (group D) received only systemic priming. Immunoglobulin A antibody titers in nasal secretions and saliva from groups A and B significantly exceeded those of groups C and D ($p < .01$). Many antigen-specific IgA-forming cells were detected in the respiratory and gastrointestinal mucosae of groups A and B. Following duodenal immunization, the appearance of antigen-specific IgA-forming cells in Peyer's patches preceded those in mesenteric and hilar lymph nodes, while they simultaneously appeared in the lymphoid tissues after tracheal immunization. These findings suggest that intratracheal immunization mainly stimulates bronchus-associated lymphoid tissue as a source of IgA precursors, as intraduodenal immunization stimulates gut-associated lymphoid tissue.

KEY WORDS — bronchus-associated lymphoid tissue, ELISA plaque-forming assay, local immunization, Peyer's patches, respiratory IgA response.

INTRODUCTION

It has been demonstrated that a considerable number of immunoglobulin A (IgA) immunocytes exist in the lamina propria of the respiratory and gastrointestinal mucosae and produce secretory IgA, which is a predominant immunoglobulin in external secretions and plays an important role in the local immune defense of the mucosal surface.[1,2] The mucosal surfaces of the respiratory and gastrointestinal tracts are thought to share a common mucosal immune system,[3] and T and B lymphoblasts from one mucosal site migrate to distant mucosal sites as well as back to their original sites.[4,5] Mucosa-associated lymphoid tissue (MALT), such as gut-associated lymphoid tissue (GALT) and bronchus-associated lymphoid tissue (BALT), is thought to be a major source of IgA immunocytes in the respiratory and gastrointestinal mucosae, since these lymphoid tissues are rich in IgA precursor cells.[6,7]

However, little attention has been paid to the effect of antigen administration on BALT, and to the factors that determine the distribution of IgA immunocytes along the respiratory tract, even though the enhancement of mucosal IgA response is very important in preventing infections and microorganic invasion of the respiratory mucosa. In an effort to clarify the exact source of IgA immunocytes in the respiratory mucosa, we attempted to induce antigen-specific IgA-forming cells in the respiratory mucosa, and to investigate the actual role of BALT in the respiratory IgA response.

MATERIALS AND METHODS

Animals. Healthy male Hartley guinea pigs 6 to 8 weeks old (250 to 300 g) were used in this experiment.

Preparation of Antigen. Dinitrophenylated ovalbumin (DNP-OVA) was prepared and characterized according to the method of Eisen et al.[8] This preparation was determined to have 7.1 DNP groups per molecule of OVA by optical density analysis.

As a particulate form of DNP-OVA antigen, DNP-OVA was conjugated with formalin-killed *Streptococcus mutans* strain 6715 (DNP-OVA–*S mutans*) according to the method described by Ternynck et al.[9] The DNP-OVA–*S mutans* had 3.2 mg of DNP-OVA per 1 mg of bacterium.

The DNP-OVA also was conjugated with fluorescein isothiocyanate (FITC) for immunofluorescent studies, by the method of Kawamura.[10]

Antisera. Antisera of rabbit against α- and γ-chain of guinea pig (Miles Scientific, Naperville, Ill) and goat anti rabbit immunoglobulin G (IgG) antisera conjugated with alkaline phosphatase (Miles Scientific), rhodamine, and horseradish peroxidase (HRP; Cappel, Novern, Pa) were purchased from commercial sources. Those labeled antisera of goat anti rabbit IgG were mixed with normal guinea pig serum to render them specific against rabbit IgG.

Immunization Schedule. Animals were injected in the hind footpads with 200 μg of DNP-OVA mixed with Freund's complete adjuvant. One week after systemic priming, 200 μg of DNP-OVA coupled with *S mutans* in 200 μL of 0.1 *M* phosphate-buffered saline was inoculated into the

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duodenal lumen under laparotomy (group A), and into the tracheal lumen by surgical exposure of the trachea (group B). Intranasal immunization (group C) was carried out through a bony window surgically made on the lateral wall of the nostril, and the particulate antigen was inoculated with 200 μL of 0.8% hydroxylpropyl cellulose solution to ensure antigenic contact with the mucosa. The control animals (group D) received a single systemic priming without local immunization.

Collection of Nasal Secretions, Saliva, and Sera. Nasal and salivary secretions and sera were collected from all animals of each group on days 10, 14, 17, and 21 after systemic priming. The secretion of the nose was stimulated by nasal drops of 10 μL of 0.1% histamine solution, and that of saliva also was enhanced by the intraperitoneal injection of pilocarpine (1.0 mg/100 g of body weight). These samples were centrifuged to remove insoluble materials, and the supernatants were used for immunochemical analyses. Blood samples for serum also were obtained by cardiac puncture at the same time.

Determinations of Antigen-Specific Antibody Titers of IgA and IgG. The indirect enzyme-linked immunosorbent assay was employed to estimate antibody titers of IgA and IgG against DNP-OVA in nasal secretions, saliva, and sera, as previously described.[11] Anti–guinea pig α- or γ-chain antiserum of rabbit was used as the first antibody, and anti-rabbit IgG antiserum of goat conjugated with HRP was employed as the second antibody.

Analysis of Antigen-Specific IgA and IgG Antibody-Secreting Cells in Various Lymphoid Tissues. Antibody-secreting cells of IgA and IgG classes against DNP-OVA in various lymphoid tissues, such as Peyer's patches, mesenteric lymph nodes (MLNs), hilar lymph nodes (HLNs) and spleen, were detected by the solid-phase immunoenzymatic technique according to the method of Sedgwick and Holt.[12] Anti–guinea pig α- or γ-chain antiserum of rabbit, as the first antibody, and alkaline phosphatase–conjugated anti-rabbit IgG antiserum of goat, as the second antibody, were used in this indirect ELISA plaque-forming assay.

Briefly, 24-well tissue culture plates were coated with DNP-OVA, as previously described.[11] One milliliter of 10^5 cells/mL cell suspension of each lymphoid tissue in RPMI 1640 plus 5% fetal calf serum was added to each well of the precoated microplates and incubated for 2 hours at 37°C. After removing the cells by washing with 0.01 *M* phosphate-buffered saline at pH 7.4, subsequent incubations were performed with rabbit anti guinea pig α- or γ-chain antiserum overnight at 4°C, followed by goat anti rabbit IgG conjugated with alkaline phosphatase. The substrate of alkaline phosphatase, 5-bromo-4-chloro-3-indolyl phosphate in 36°C gelling agarose, was added to each well and incubated overnight at 4°C to yield insoluble blue products. All blue spots were counted in each well under 40x magnification by use of an inverted microscope.

Identification of Antigen-Specific IgA-Forming Cells in Various Tissues. To identify antigen-specific IgA-forming cells in various tissues, the double staining technique was employed according to the method of Kawamura.[10]

Fresh tissue specimens were either frozen over dry-ice ethanol for frozen section, or fixed in 95% cold ethanol for paraffin section. The 5-μm serial sections were treated with Lendrum's phenol chromotrope 2-R stain prior to immunofluorescent staining to abolish nonspecific fluorescent staining of granulocytes.[13]

To stain specific antibody-forming cells against DNP-OVA, sections were incubated with FITC-labeled DNP-OVA overnight at 4°C and, after washing with 0.01 *M* phosphate-buffered saline, observed under a 495-nm blue exciting beam with a 510-nm barrier filter specific for FITC. Nonspecific binding of FITC-labeled DNP-OVA was blocked by pretreatment of consecutive sections with FITC unconjugates or bovine serum albumin prior to staining with FITC conjugates of DNP-OVA. Immunoglobulin A–forming cells were stained by the indirect technique with anti–guinea pig α-chain antiserum of rabbit as the first antibody, and anti-rabbit IgG antiserum of goat conjugated with rhodamine as the second antibody. Rhodamine conjugates of IgA-forming cells were observed under a 546-nm green exciting beam with a 590-nm barrier filter. Nonspecific binding of anti–guinea pig antisera of rabbit was blocked by incubation with normal rabbit serum prior to addition of anti–guinea pig α-chain of rabbit. Blocking tests were performed by supplying appropriate unconjugated antibodies to consecutive tissue sections prior to staining with rhodamine conjugates.

RESULTS

Antigen-Specific Antibody Titers of IgA and IgG in Nasal Secretions, Saliva, and Sera. Mean titers of specific antibody of IgA and IgG classes against DNP-OVA in nasal secretions, saliva, and sera from each group 2 weeks after the initial systemic immunization are illustrated in Fig 1.

The mean values of IgA antibody titers in nasal secretions and saliva from animals of groups A and B with intraduodenal or intratracheal immunization were significantly greater than those of groups C and D ($p < .01$), while serum IgG antibody titers of groups C and D significantly exceeded those of groups A and B ($p < .01$). However, there were no statistically significant differences between the antibody titers of groups C and D.

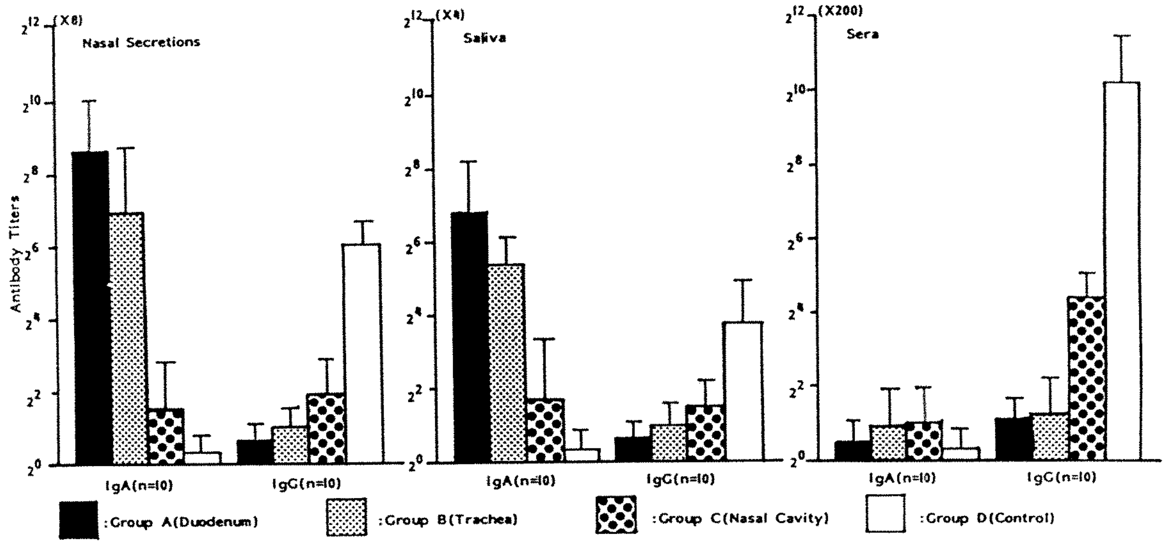

Fig 1. Antigen-specific antibody titers of immunoglobulin A (IgA) and immunoglobulin G (IgG) in nasal secretions, saliva, and sera. Columns indicate mean values of antibody titers from animals of each group, and half-brackets show standard deviation of mean.

Kinetics of Antigen-Specific Antibody Titers of IgA and IgG in Nasal Secretions and Saliva. Animals received local immunization with DNP-OVA-*S mutans* in the duodenum, trachea, or nasal cavity 1 week after systemic priming. On days 3, 7, 10, and 14 after local inoculation, nasal secretions and saliva were collected, and their specific antibody titers of IgA and IgG were measured. Figure 2 shows the kinetics of antigen-specific antibody titers of IgA in nasal secretions and saliva after local immunization.

After intratracheal immunization, the peak level of antigen-specific IgA antibody titers in nasal secretions was on day 7, followed by that in saliva, which was on day 10. On the other hand, IgA antibody titers in nasal secretions after intraduodenal immunization slowly rose and reached the peak level on day 10. The peak of salivary IgA antibody titers, which was on day 7, preceded that of nasal secretions.

Cell Kinetics of Antigen-Specific IgA Antibody-Secreting Cells in Various Lymphoid Tissues. Figure 3 illustrates cell kinetics of antigen-specific IgA-secreting cells in Peyer's patches, MLNs, HLNs, and spleen after local immunization into the duodenum, trachea, and nasal cavity with DNP-OVA-*S mutans*.

The intraduodenal immunization elicited a remarkable increase of antigen-specific IgA-secreting

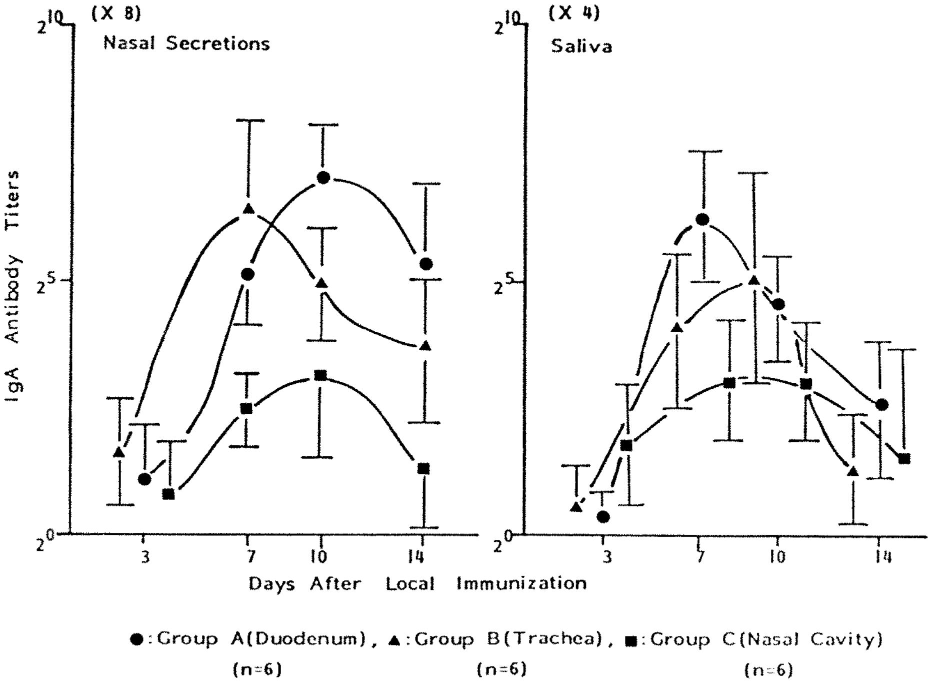

Fig 2. Kinetics of antigen-specific antibody titers of immunoglobulin A (IgA) in nasal secretions and saliva from animals of each group. Each group contained six animals; geometric mean values are expressed. Brackets indicate standard deviation of mean.

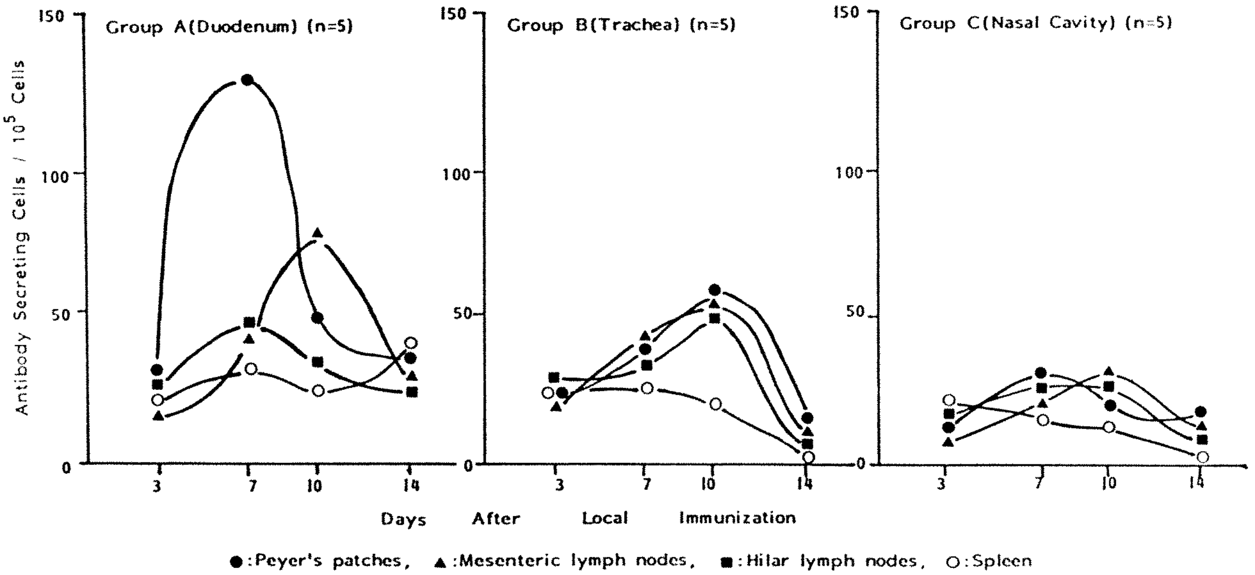

Fig 3. Cell kinetics of antigen-specific immunoglobulin A antibody-secreting cells in various lymphoid tissues from animals of each group. Each group contained five animals, and geometric means of each group are shown.

cells in Peyer's patches ($p < .01$), and the peak cell number was on day 7 after local immunization. That was followed by the elevation of antigen-specific IgA-secreting cells in MLNs, of which the peak level was on day 10. In contrast, the intratracheal immunization resulted in parallel increases of IgA antibody-secreting cells in Peyer's patches, MLNs, and HLNs, and reached the peak level on day 10 after local immunization.

Distribution of Antigen-Specific IgA-Forming Cells in Various Tissues. Figure 4 summarizes the distribution of antigen-specific IgA-forming cells. A considerable number of these IgA-forming cells were detected along the respiratory mucosa, such as the nasal mucosa (Fig 5A,B), nasopharynx (Fig 5C,D), and trachea (Fig 5E,F), as well as the gastrointestinal tract after local immunization into the duodenal or tracheal lumen. However, these cells could not be found in the mucosa of the respiratory and gastrointestinal tracts of group C animals with intranasal immunization. Many IgA-forming cells against DNP-OVA, which were stained with both FITC and rhodamine conjugates, were observed to be scattered in the subepithelial connective tissue between the glands of nasal mucosa and other respiratory mucosae. The number of the cells was smaller than that of IgA-forming cells that were detected with rhodamine-conjugated anti-guinea pig α-chain. Following intratracheal immuniza-

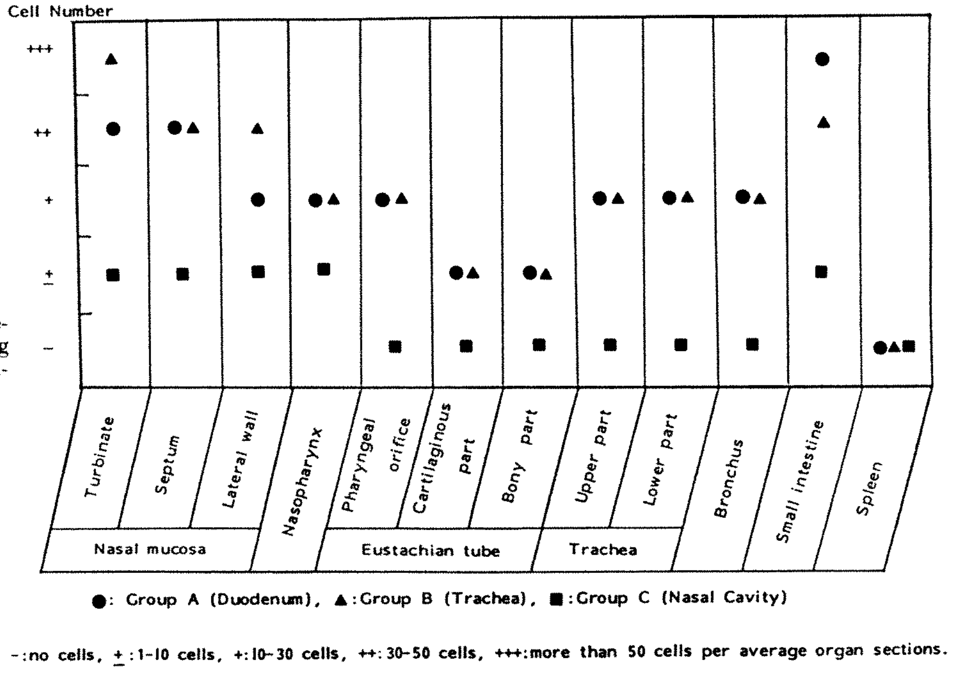

Fig 4. Distribution of antigen-specific immunoglobulin A-forming cells in various tissues from animals of each group.

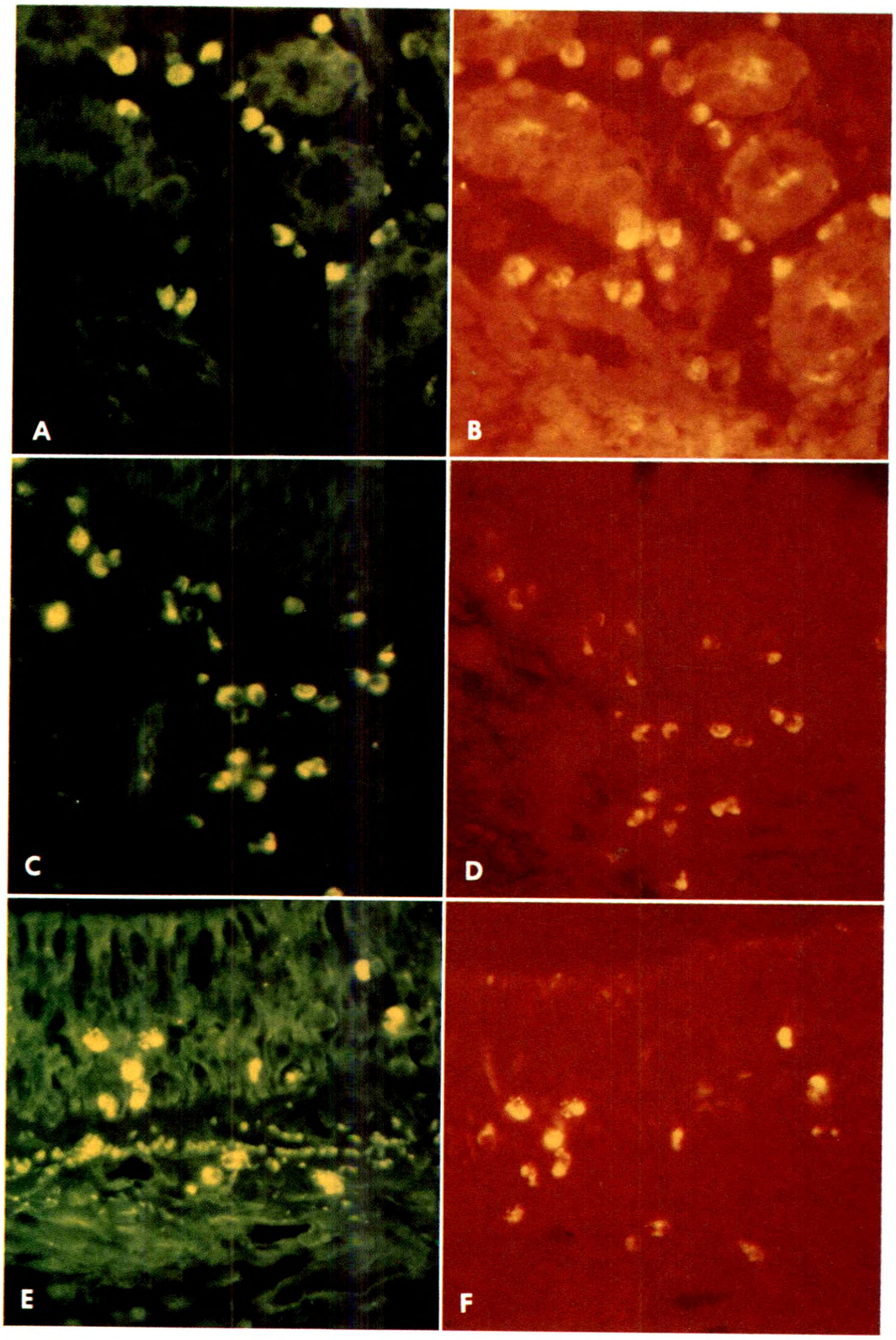

Fig 5. Distribution of antigen-specific immunoglobulin A (IgA)–forming cells in A,B) nasal mucosa, C,D) nasopharynx, and E,F) trachea from animals with intratracheal immunization. Tissue sections of A, C, and E were stained with fluorescein isothiocyanate (FITC) conjugates for antigen-specific antibody-forming cells, and those sections of B, D, and F were treated with rhodamine conjugates for IgA-forming cells. Antigen-specific IgA antibody–forming cells exhibit fluorescences of both FITC and rhodamine.

tion, these antigen-specific IgA-forming cells seemed to be more abundant in the respiratory mucosa than in the mucosae from animals with intraduodenal immunization.

DISCUSSION

The present study demonstrates the effective induction and activation of the respiratory IgA immune response by the mucosal immunization with the particulate antigen into the duodenal or tracheal lumen after systemic priming with the soluble form. The appearance of antigen-specific IgA plasma cells in the respiratory mucosa after the duodenal or tracheal immunization is considered further evidence for a common mucosal immune system and directly proves that the respiratory mucosa shares common mucosal immunity with the gastrointestinal mucosa. These IgA-forming cells in the respiratory mucosa are thought to be derived from MALTs, such as GALT and BALT, since these lymphoid tissues are rich in precursors of IgA plasma cells.[6,7] After antigenic stimulation of GALT or BALT, precursors committed to antigen-specific IgA-forming cells enter the circulation through the thoracic duct, differentiate while circulating, and finally settle in the mucosa of the antigen-stimulated organ itself and distant secretory sites. However, the intranasal immunization, in comparison with the intraduodenal or intratracheal immunization, was not an effective route for inducing respiratory IgA response, even though care was taken to ensure that antigen remained in contact with the mucosa of the nasal cavity for as long as possible. It is likely that some antigen was ingested following mucociliary clearance on the nasal mucosa and lost its antigenicity because of digestion and intragastric immunization with protein antigens.[14,15] Furthermore, the nasal mucosa appears to have no associated lymphoid tissues similar to GALT and BALT. Therefore, the intranasal immunization is not effective in producing a respiratory IgA response.

Although the effect of oral immunization stimulating GALT on the local IgA response of the gastrointestinal tract has been well documented, little attention has been paid to the induction and actual process of respiratory IgA immune response. The role of BALT in producing IgA-secreting cells is unclear, even though it is assumed that BALT is involved in the immune defense of the respiratory tract because of its localization and morphologic similarity to GALT.[7,16,17] Previous investigation suggested that IgA response of the respiratory tract in animals with poorly developed BALT can be enhanced by harnessing the immune potential of GALT, as the largest source of IgA precursors, by appropriate immunization strategies.[18] However, Plesch et al[19] have demonstrated that after intratracheal stimulation, the epithelium overlying BALT becomes infiltrated with lymphocytes and forms a lymphoepithelium, although the epithelium has cilia in unstimulated animals and is rarely infiltrated with lymphocytes. Stein-Strelein and Hart[20] suggested that GALT is not the source of IgA-secreting cells in the respiratory mucosa and lymph nodes, since the IgA-secreting cells' response in these lymph nodes was not altered significantly by intestinal lymph drainage, and that the IgA immune response of the respiratory tract is probably attributable to direct antigen stimulation of BALT. The results of the present study are consistent with those findings.

In this experiment, guinea pigs were used as experimental animals, and these animals are known to have extensively distributed BALT.[7] When the particulate antigen is administered on the mucosal surface of the respiratory tract, it may be trapped selectively by M cells overlying BALT.[21] In this study, following intratracheal immunization, the response of IgA antibody titers in nasal secretions preceded that in saliva, and the appearance of IgA antibody–secreting cells in Peyer's patches was similar to those in MLNs and HLNs. Intraduodenal immunization resulted in the sequential appearance of peak IgA-secreting cell responses in the particular order in which the peak response in Peyer's patches was followed by that in MLNs. These findings indicate that after tracheal immunization, these lymphoid tissues and mucosae of the respiratory and gastrointestinal tracts are supplied with IgA-secreting cells from a single source different from Peyer's patches. The source of these IgA-forming cells is probably BALT.

Although guinea pigs are known to possess extensively distributed BALT, it is not clear that these animals have palatine tonsils and adenoid tissues like those in humans. Human palatine tonsil has been demonstrated to have specialized epithelial cells with microvilli in the microcrypt.[22] These specialized cells seem to be similar to M cells of GALT and BALT, and they play an important role in trapping and transport of antigenic substances.[23,24] These morphologic and histologic similarities of human palatine tonsils and adenoids may suggest that their functions in local immune response are similar to those of GALT and BALT.

Mucosal immunization results in the appearance of antigen-specific IgA-forming cells in various mucosae of the respiratory and gastrointestinal tracts. However, the mechanisms responsible for the selective localization of these IgA-forming cells are still unknown, and this localization occurs irrespective of antigenic stimulation on the local site. Hormonal factors[25] and the secretory component[26] may influence the emigration of IgA precursors to secretory sites. Recently, the interactions between various lymphocyte populations and high endothelial venules, which are specialized vessels of lymphoid organs and major sites of lymphocyte emigration from the bloodstream, have been demonstrated.[27] The results of the present study suggest that IgA-forming cells originating from BALT by intratracheal immunization preferentially appear in the respiratory tract, while those from GALT by intraduodenal immunization prefer to home to the gastrointestinal tract. This migration of IgA precursors from GALT or BALT may be attributed to local antigenic stimulation or to the difference between mucosal and peripheral endothelial cell determinants of high endothelial venules.

In conclusion, the data presented here provide further evidence for a common mucosal immunity and establish a principle of immunization strategy whereby respiratory local immunity can be enhanced. Local immunization into the tracheal lumen mainly stimulates BALT as a source of IgA precursors, much as intraduodenal immunization stimulates GALT, and results in the appearance of antigen-specific IgA-forming cells in the respiratory mucosa. This correlates with an increase of IgA antibody titers in external secretions, such as nasal and salivary secretions, for protective immunity of the respiratory tract.

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SELECTIVE REINNERVATION OF THE ABDUCTOR AND ADDUCTOR MUSCLES OF THE CANINE LARYNX AFTER RECURRENT NERVE PARALYSIS

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Functional rehabilitation of the larynx after unilateral vocal cord paralysis was attempted in the dog by selective reinnervation of the laryngeal muscles. The intralaryngeal branches of the right recurrent nerve were dissected. The adductor branch was anastomosed with the ansa cervicalis; the abductor branch was anastomosed with the trunk of the phrenic nerve either within the larynx or through the recurrent nerve, the adductor branch of which was sectioned. Results could be analyzed in seven dogs: mobility of the vocal cord was checked, and electromyography, stimulation of the nerves, and histologic studies were performed. Functional reinnervation of both the adductor and abductor muscles was obtained in only one case, with good abduction. Adduction was recorded in five cases. False-positive results emphasize the necessity of collecting several types of data before concluding that functional reinnervation has been accomplished. The reliability of the procedure can and must be improved.

KEY WORDS — experimental surgery, laryngeal muscles, reinnervation, vocal cord paralysis.

INTRODUCTION

Bilateral recurrent laryngeal nerve paralysis constitutes a problem that is still only incompletely solved. Indeed, both vocal cords usually lie in a paramedian position that may result in inspiratory dyspnea. However, phonation is preserved satisfactorily. Classical modes of treatment for bilateral paralysis are aimed at enlarging the glottis; hoarseness and lack of protective closure result with most of them. Laryngeal reinnervation might solve these problems.

Since the end of the last century, many attempts have been made, in both animals and humans, either to perform direct neurorrhaphy of the sectioned recurrent nerve or to anastomose its distal trunk with a cervical motor nerve. Most of them failed to show vocal cord movement. An explanation was suggested by Siribodhi et al[1] in 1963: the electrical activity recorded in the reinnervated laryngeal muscles without movement of the vocal cords could result from the misdirected regeneration of the motor axons to the adductor and abductor muscles. This hypothesis has been confirmed by electromyographic (EMG) studies by Dedo and Ogura,[2] Dedo,[3] Miehlke et al,[4,5] and Sato and Ogura.[6] Recovery of either adductor or abductor function was the subject of subsequent experiments. Fex[7] was the first to obtain functional reinnervation of the posterior cricoarytenoid (PCA) muscle in the cat with direct implantation of the phrenic nerve. Taggart[8] and Morledge et al[9] reported similar results in the dog.

In 1970, Tucker et al[10] reported a new technique with success in the dog: this was the neuromuscular pedicle used to reinnervate the PCA muscle with the ansa cervicalis nerve. This technique has been the subject of many experimental studies[11-14] and has been performed in human patients by Tucker[15,16] in a large number of cases described as successful. But some authors were not so satisfied and some visual and histologic evidence was lacking: Neal et al,[17,18] Crumley,[19] and Rice et al[20] showed a lack of reinnervation of the PCA muscle with this method in their experiments. Nevertheless, recent studies by Anonsen et al[21] and Meikle et al[22] seem to provide histologic proof of reinnervation by the neuromuscular pedicle technique.

At the same time, other authors were interested in selective anastomosis of motor nerves with branches of the recurrent nerve. The first experiments dealt with the abductor branch of the PCA muscle, which was anastomosed with the phrenic nerve by Murakami and Kirchner,[23] Miehlke et al,[24] Miehlke,[25] Crumley,[19] and Rice.[26] Then anastomosis was performed with both abductor and adductor branches simultaneously: Iwamura[27] in 1974, Miehlke et al[28] in 1981, and Crumley[29] in 1984, with interesting results. This is the setting for the present study. It was aimed at confirming the feasibility and utility of selective reinnervation of the laryngeal muscles with the phrenic nerve and the ansa cervicalis as proposed by Crumley.[29]

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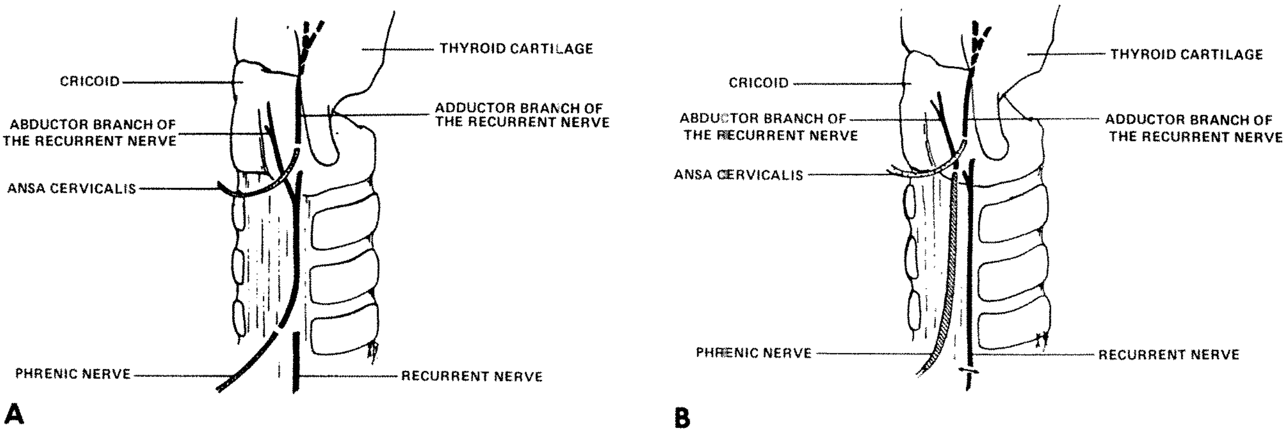
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Fig 1. Techniques used for anastomosis between phrenic nerve and abductor branch of recurrent nerve. Adductor branch is anastomosed with ansa cervicalis. A) Technique A. Extralaryngeal anastomosis. B) Technique B. Intralaryngeal anastomosis.

METHODS

Eleven dogs weighing from 10 to 15 kg were operated on with use of general anesthesia with Fluothane. Orotracheal intubation was used and the animal breathed spontaneously. A medial cervical incision was made from the hyoid bone to the sternum. On the right side were identified the recurrent nerve, ansa cervicalis, and phrenic nerve. The right recurrent nerve was sectioned at the level of the fourth cervical ring. After section of the cricopharyngeal muscle, identification of the terminal branches of the recurrent nerve was carried out under an operating microscope. The ansa of Galen between the recurrent and internal laryngeal nerves was resected. The abductor branch of the recurrent nerve was located in a posteromedial position. The anterolateral adductor branch was sectioned near its origin and anastomosed with the ansa cervicalis (the sternohyoid branch was most often used for convenience). The abductor branch was anastomosed with the phrenic nerve trunk by use of two techniques (Fig 1): 1) an extralaryngeal technique whereby all branches of the distal trunk of the recurrent nerve were sectioned except the abductor branch (technique A, used on seven dogs) and 2) an intralaryngeal technique directly connecting the abductor branch to the proximal trunk of the phrenic nerve (technique B, used on four dogs). A very low, slightly intrathoracic dissection and section of the phrenic nerve was necessary to permit its anastomosis with the abductor branch of the recurrent nerve (with resection of the last cervical root in a few cases).

The anastomoses were performed with four or five epineurial stitches of nylon or resorbable 10-0 suture material. The wound was closed and drained. When the dog awoke, laryngoscopic inspection of the immobility of the right vocal cord was performed.

At 3 to 6 months, direct laryngoscopy was carried out (with use of light anesthesia with intravenous thiopental) to evaluate the mobility of the experimental vocal cord. When the animal was awake, sequential photographs of vocal fold movement were made. During the same session, laryngeal EMG was performed with a bipolar needle electrode inserted successively in the PCA muscle and the thyroarytenoid muscle to observe EMG activity during deep inspiration and whining, induced respectively by tracheal obstruction and painful stimulation.

At 8 months, a video recording of laryngeal movement was carried out through a direct suspension laryngoscope.

At 9 months, the dogs underwent a cervicotomy that made possible

1. Identification of the nerve.
2. Electrical stimulation of the nerves to check the mobility of the vocal cord.
3. Observation of the larynx during obstructive dyspnea after section of the strap muscles and of the external branch of the superior laryngeal nerve.
4. Removal of the nerves and anastomosis of the abductor and adductor muscles and of the cricoarytenoid joint for histologic studies (hematoxylin and eosin stain, supplemented by Bodian silver method and the Luxol fast blue method for nerves; for comparison, both right and left muscles were studied with standard stains, and acetylcholinesterase activity at the myoneural junction was also studied).

RESULTS

Four dogs died before they could be studied: one during the operative procedure, another from a kennel epidemic 1 week after operation, and two from other diseases. None of the dogs showed symptoms of aspiration or inspiratory dyspnea.

The isolated recovery of mobility or recording of electrical activity in the laryngeal muscles is not suf-

SUMMARY OF RECORDED DATA

Dog	*Technique**	*Abduction/ Adduction*	*Mobility of Reinnervated Vocal Cord*	*Signs of Reinnervation on EMG*	*Movement of Right Vocal Cord Induced by Nerve Stimulation*	*Healthy Appearance of Nerve and Anastomosis During Cervicotomy*	*Healthy Histologic Appearance of Anastomosis and Distal Nerve*	*Healthy Histologic Appearance of Effector Muscle*
1	A	ABD	–	+	+	+	±	±
		ADD	+	±	+	+	+	+
2	A	ABD	+ +	+	+	+	+	+
		ADD	+	±	+	+	+	+
3	A	ABD	±	±	+	+	±	±
		ADD	–	±	+	+	±	+
4	A	ABD	+	+	NS	–	NS	+
		ADD	+	±	+	+	+	+
5	B	ABD	+	±	NS	–	NS	+
		ADD	+	+	+	+	+	+
6	B	ABD	+	+	–	–	–	–
		ADD	±	±	+	+	+	+
7	B	ABD	±	±	±	+	†	†
		ADD	+	+	+	+	†	†

From Marie.[30] Results — excellent (+ +), satisfactory (+), uncertain (±), or failure (–); NS — nerve not seen.

*See text for definition.

†For technical reasons, no histologic study was performed.

ficient to prove that reinnervation has occurred or is functional. In our study, the functional character of the reinnervation was demonstrated by a series of tests, specifically

1. Demonstration of the mobility of the right vocal cord during dyspnea and phonation.
2. Electrical indices of reinnervation recorded in the muscle (simple detection) and mobility of the vocal cord after electrical stimulation of the nerve.
3. Macroscopic observation of nerves and anastomosis (cervicotomy) and histologic findings.

The various findings recorded for each dog are compiled in the Table.[30]

Recovery of mobility was observed: the abduction of the operated vocal cord was greater than or equal to 60% of the mobility of the opposite vocal cord in four dogs (Nos. 2, 4, 5, 6). Adduction was greater than or equal to 70% in five dogs (Nos. 1, 2, 4, 5, 7).

Electrical signs of reinnervation were the recording of recruitment of motor units and modification of the shape of the action potentials so that they were prolonged and polyphasic.

When cervicotomies were performed, the phrenic nerves were not found in dogs 4 and 5. In dog 6, the phrenic nerve terminated in the cricopharyngeal muscle.

Histologic study was aimed at counting the axonal population proximal and distal to the anastomosis, studying the anastomosis, and determining the presence of muscular atrophy. Escape of small groups of axons from within the epineurium was observed in several histologic sections of the anastomosis or the distal nerve, even in the abductor anastomosis of dog 2.

Thus, the results can be reported as follows:

1. Of the seven dogs, we were able to demonstrate functional reinnervation of the abductor (PCA muscle) in one dog (No. 2, extralaryngeal technique A).
2. Functional reinnervation of the adductors was obtained in five dogs (Nos. 1, 2, 4, 5, 7).
3. Recovery of both abductory and adductory functions was obtained in only one dog (No. 2, extralaryngeal technique A) (Figs 2-4).

In dog 2, during quiet respiration, when the phrenic nerve was usually active, but not the recurrent nerve, there was abduction only of the reinnervated right vocal cord. This shows quite clearly that the PCA muscle had been reinnervated by the phrenic nerve. The reinnervated vocal cord was abducted a little later than the healthy side during respiration, following the known time lag between PCA muscle activity and that of the diaphragm.

Abductory function did not recover in the three dogs with phrenic intralaryngeal anastomosis (technique B).

Reinnervation of the adductor muscles was demonstrated, but its functional character was not proved; adductory movements were obtained but their effects on phonation were not studied.

Recovery of one of the two functions did not exclude recovery of the other one.

Section of both superior laryngeal nerves and of the strap muscles decreased adductory, but not abductory mobility.

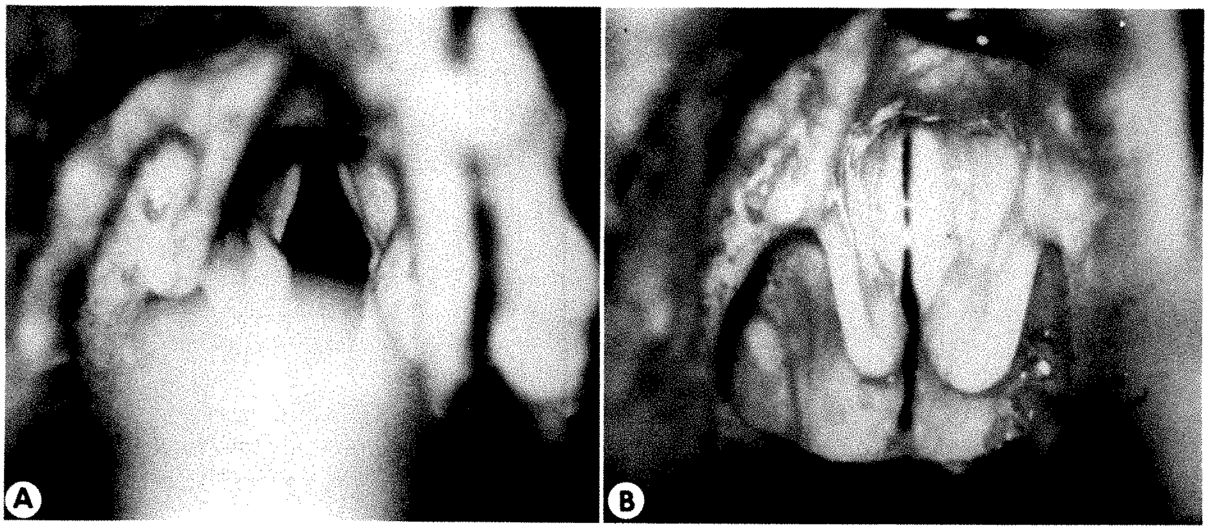

Fig 2. (Dog 2) Laryngoscopic views 8 months after procedure. A) Deep inspiration. B) Whining. Note position of reinnervated vocal fold (right side).

The chronology of reinnervation could not be measured precisely. However, to judge from laryngoscopic observations, recovery of adduction and abduction was essentially complete within 3 months in dog 2.

The results obtained in dog 2 did not deteriorate during the 10-month period of the study. On the other hand, in dog 7, abduction seemed to deteriorate between 4 and 5 months. This phenomenon also has been observed by Crumley[19] and could be explained by the erroneous reinnervation of a few adductory muscle fibers by axons that escaped from the abductory branch to the PCA muscle. We were not able to observe either early electrical signs of reinnervation before recovery of mobility or improvement of this movement from 3 to 6 months (cited by Iwamura[27]).

DISCUSSION

The reasons for failure of a given anastomosis may provide guidance for the improvement of operative technique. However, in our series the reasons could be determined in only two animals.

In dog 6 (technique B), in spite of slight abductory movement, the phrenic nerve was found to terminate in the cricopharyngeal muscle. It is possible that the anastomosis was broken because of tension, and axonal sprouts from the phrenic nerve reached the wrong muscle.

In dog 3 (technique A), the reinnervated vocal cord did not reach the midline during whining. Nevertheless, the adductor muscles were reinnervated, since adduction of the right vocal cord was obtained by electrical stimulation of the ansa cervicalis and no atrophy was found. This apparent contradiction may be explained by the following observation: during extended obstructive dyspnea (after section of the strap muscles and of the external branch of the superior laryngeal nerve), it was the only case in which paradoxical adductory movement was observed, and this movement was interrupted by section of the ansa cervicalis. Indeed, the sternohyoid muscle (like the other strap muscles except possibly the thyrohyoid muscle[29]) is activated during inspiratory obstructive dyspnea. Hence, firing of the ansa cervicalis resulted in untimely contraction of the adductor muscles. Both abductory and adductory movements were unsatisfactory. This observation constitutes an argument against use of the ansa cervicalis to reinnervate the adductor muscles. On the other hand, one can conclude, in agreement with Crumley,[29] that the thyrohyoid branch should be selected.

Probable reasons for failure of the procedure are 1) a defect of the anastomosis (tension or infection), 2) poor vascularization of the nerve after grafting, 3) variations of the intralaryngeal twigs of the recurrent nerve, and, above all, 4) axonal escape from the anastomosis. Histologic findings often revealed epineurial axonal escape around the distal (especially phrenic) nerve. These regenerated axons may have reinnervated the antagonistic muscles. This hypothesis may explain the lack of inspiratory abduction observed in dog 1. However, abductory movement was obtained by stimulation of the proximal trunk of the phrenic nerve. Inspiratory EMG data from the PCA muscle showed signs of reinnervation; unfortunately, EMG data from the thyroarytenoid muscle during inspiration were not recorded.

In technique B, there was a mismatch between the diameters of the abductor branch of the recurrent nerve and the phrenic nerve that contributed to failure of the anastomosis.

Another consequence of axonal escape is early oc-

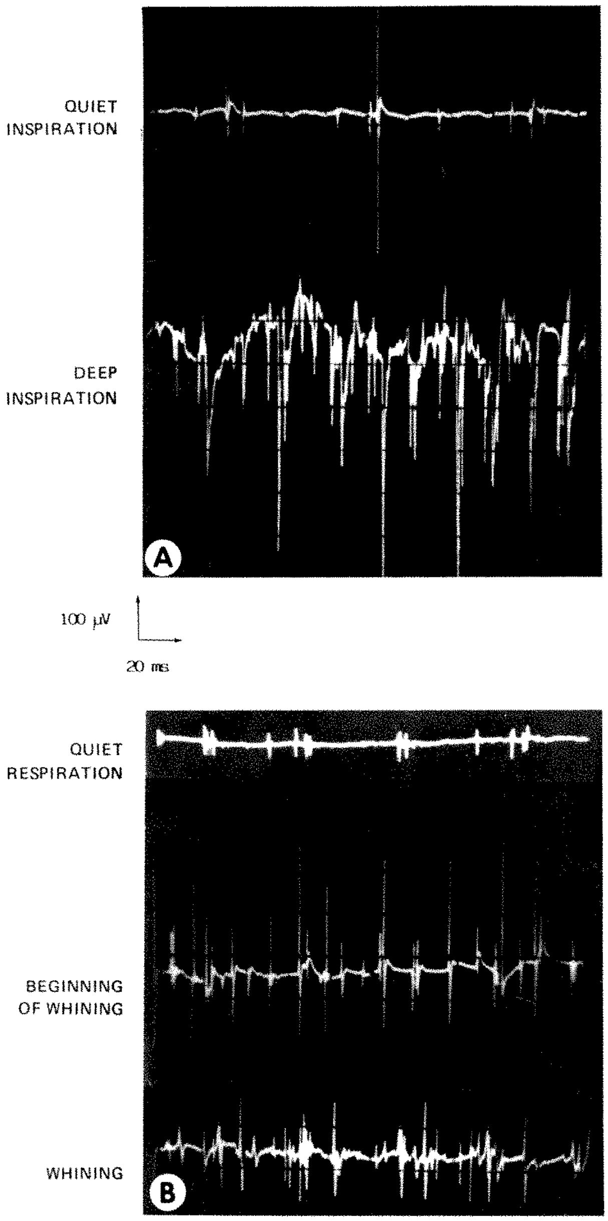

Fig 3. (Dog 2) Electromyograms of reinnervated side recorded 3 months after procedure. A) Right posterior cricoarytenoid muscle abduction. B) Right thyroarytenoid muscle adduction.

cupation of the wrong motor end plates, preventing reinnervation by another nerve fiber.[19]

All of these facts emphasize that it is necessary to avoid axonal escape, using, for example, a nerve graft to fit the phrenic and abductor stumps and a sheathed anastomosis.

Ankylosis of the cricoarytenoid joint was never observed, despite the prolonged immobilization of the arytenoid in the unsuccessful cases.

Certain observations should be made in regard to false-positive results. In dog 6, a lateralization of the right vocal cord was observed without reinnervation of the PCA muscle, which was found to be atrophic. Although there was no ankylosis of the cricoarytenoid joint, this lateralization may have resulted from posterior and lateral fixation of the arytenoid by postoperative scarring of the PCA muscle and by scar tissue linking the PCA muscle to the cricopharyngeal muscle, which may have been partly reinnervated by the phrenic nerve.[17,19,20] We also should mention the effect of the contraction of strap muscles, which results in lateralization of the arytenoid cartilages and the vocal folds, and the effect of cricothyroid muscle contraction, which increases the area of glottal opening by lengthening the vocal folds.[31]

The contraction of the same cricothyroid muscle can result in a movement equivalent to 35% to 45% of the contralateral adductory movement.[27,32] In this study, the external branches of the superior laryngeal nerves were sectioned only after the second cervicotomy.

Analysis of failures and false-positive results leads us to emphasize the necessity of comparing the different data collected, especially EMG and histologic findings, in order to assess the outcome of experiments in reinnervation.

The nerves used in this study were the phrenic nerve and the ansa cervicalis. The inspiratory nature of the phrenic nerve makes it an ideal source for reinnervation of the PCA muscle, as confirmed by recent experiments.[19,24,26-29,33-35] The results obtained seem better with the phrenic nerve than when the ansa cervicalis is anastomosed to the abductor branch of the recurrent nerve.[34,36] Although it is the main motor nerve to the diaphragm, transection of the phrenic nerve seems to be remarkably well tolerated by humans.[37,38]

Unfortunately, we were unsuccessful with the technique of intralaryngeal anastomosis, which was most similar to the conditions that might be encountered in patients (because of unavailability of the injured recurrent nerve). It seems that use of a nerve graft cannot be avoided.

The ansa cervicalis was able to reinnervate the adductor muscles. Both atrophy and also misdirected reinnervation by axons from the abductory anastomosis were thus avoided, although probably not in all cases. Adductory movement was effective in the five successful operations; use of the thyrohyoid branch of the ansa cervicalis should give better results. We could not analyze the effect of adduction and lack of atrophy, ie, preservation of the muscle mass that gives shape to the vibrating mucosa in phonation. But one can suppose that both these effects of reinnervation provide an improvement of the voice.[16,39-41] This idea requires further study.

It would also be important to consider the ability of the reinnervated larynx to close after supraglottic

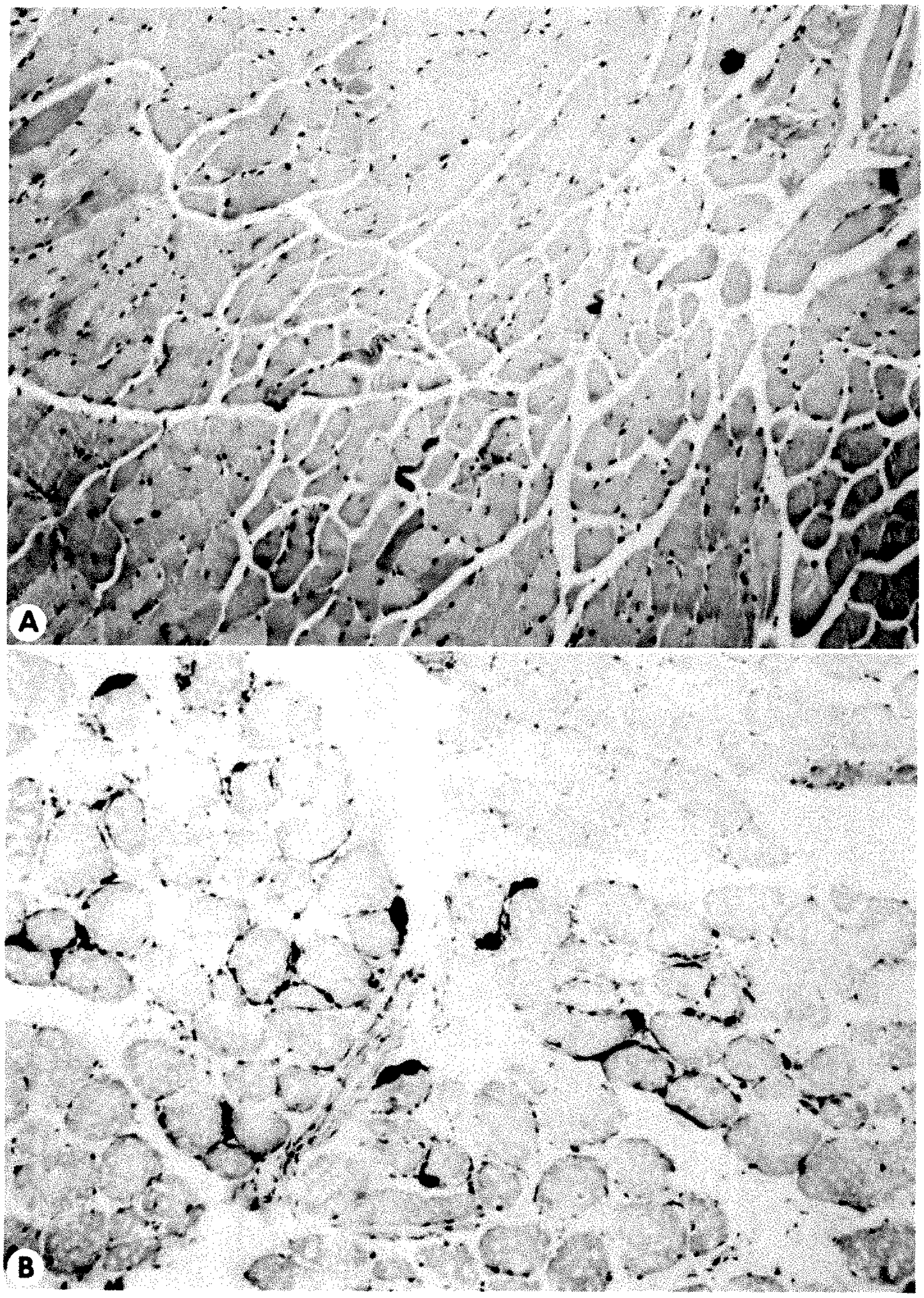

Fig 4. (Dog 2) Reinnervated posterior cricoarytenoid muscle. A) Standard stain; note lack of atrophy. B) Histoenzymatic study (acetylcholinesterase activity); note great number of myoneural junctions.

stimulation. Would the ansa cervicalis be able to conduct efferent impulses for reflex closure? With what delay?

However, improvement in the reliability of recovery of abductory movement is necessary before these questions can be addressed.

SUMMARY AND CONCLUSIONS

Functional rehabilitation of the larynx after unilateral recurrent nerve section was attempted by use of the phrenic nerve trunk and the ansa cervicalis in order to reinnervate the PCA muscle and the adductor muscles. Of the seven surviving dogs, recovery of both abductory and adductory functions was obtained in only one, with good abduction. Adduction was recovered in five. The discovery of false-positive results emphasizes the necessity of collecting several types of data before concluding that functional reinnervation has been accomplished.

These results must be improved. Nevertheless, the good quality of the recovered mobility, taken together with the success of other published studies, seems to justify further experiments. They should be aimed at reliable recovery of abduction and adduction with the best possible adaptation for phonation and laryngeal protection.

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HIGH ANTIBODY LEVELS AGAINST MOUSE LAMININ WITH SPECIFICITY FOR GALACTOSYL-(α1-3)GALACTOSE IN PATIENTS WITH INNER EAR DISEASES

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Sera of 413 patients with inner ear diseases were examined for ELISA binding to mouse laminin and compared to a normal group. Strongly enhanced antilaminin antibody reactions were observed in a large number of patients with sensorineural hearing loss (68%), tinnitus (60%), and sudden deafness (46%), but not in those with Meniere's disease (14%) or normal individuals (8%). Absorption experiments demonstrated that these antibodies are also responsible for the antisarcolemmal antibody pattern in the immunofluorescence test and for the reaction with pig kidney microsomes in the ELISA. Immunochemical studies showed that laminin binding of high or low titer in sera from patients and normal individuals is due to the Galα1-3Gal epitope present in N-linked oligosaccharides of mouse laminin. No reactions were observed with human laminin, which lacks this epitope. The findings suggest that high levels of antibodies against carbohydrate structures are triggered by persistent infections, providing some new insights about the possible cause of these diseases.

KEY WORDS — antibody, galactosyl-(α1-3)galactose, inner ear disease, mouse laminin.

INTRODUCTION

Sensorineural hearing loss, sudden deafness, tinnitus, and Meniere's disease are different clinical expressions of inner ear diseases whose etiopathogenesis is unknown. Viral infections and autoimmune processes have been considered as being involved.[1-5] An association has been found with other systemic chronic inflammatory disorders such as arthritis, vasculitis, and Cogan's syndrome.[4,6] In previous studies we provided evidence for the occurrence of antisarcolemmal antibody and antiendothelial antibody in the immunofluorescence test,[7] and it could be shown that antisarcolemmal antibody also had specificity for antigens associated with the endoplasmic reticulum.[6]

A tissue reaction pattern similar to that seen for antibodies from patients with inner ear diseases has been described for antibodies from patients with acute or chronic Chagas' disease, which arises as a consequence of trypanosomal infections.[8-10] Subsequent studies demonstrated that most of these antibodies bind to mouse laminin,[10] which is an ubiquitous basement membrane protein.[11] Laminin-binding antibodies also have been detected in a variety of unrelated disorders such as American cutaneous leishmaniasis,[12] poststreptococcal glomerulonephritis,[13] and preeclampsia.[14] These antibodies also showed an unexpected cross reaction between different basement membrane proteins,[15,16] erythrocytes,[12,15] and parasites,[17] indicating that the epitope(s) involved represent(s) a common posttranslational modification, for example a carbohydrate structure. This epitope was identified recently as a terminal galactosyl-(α1-3)galactose group present on mouse but not human laminin.[16] Antibodies of identical or similar specificity are ubiquitous in the normal human population[18,19] and also can be detected by laminin binding.[16] These natural antibodies occur, however, with distinctly lower titers than in various patients, allowing a discrimination based on enzyme-linked immunosorbent assay (ELISA) or radioimmunoassay (RIA).[10,12,13,15] The antigenic stimuli responsible for generating the natural antibodies may be quite variable,[18,19] while carbohydrate epitopes shared by mouse laminin and some specific parasites[16] are likely to augment the response in certain tropical diseases.

On the basis of these observations we considered the possibility that patients with inner ear diseases also may have elevated levels of antibodies against the Galα1-3Gal epitope. In the present study we demonstrated a high titer of these antibodies in laminin-binding assays and verified their carbohydrate specificity in immunochemical studies. These data shed some new light on the possible etiopathogenesis of these diseases and also may be useful for their prognosis.

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PATIENTS

Sera were collected from 413 patients with different forms of inner ear diseases (mean age, 35.2 years; standard deviation, 15.1; range, 12 to 65 years; male to female ratio, 2:1); 296 patients had chronic sensorineural hearing loss of unknown cause, 48 patients had sudden deafness, 49 patients had Meniere's disease, and 20 patients had tinnitus.

As controls we tested sera from 250 patients with defined antinuclear antibody-positive or -negative collagen disorders (mean age, 31.3 years; standard deviation, 17.3; range, 15 to 72 years; male to female ratio, 1:5) and from 270 patients with autoimmune chronic active hepatitis (mean age, 41.7 years; standard deviation, 17.9; range, 6 to 82 years; male to female ratio, 1:2).

Sera from 100 blood donors who were matched by age and sex to the patients with inner ear disorders (mean age, 33.3 years; standard deviation, 15.8; range, 18 to 53 years; male to female ratio, 2:1) were included as normal controls in the study. All sera were stored at −20°C.

METHODS

Antigens and Haptens. Laminin from the Engelbreth-Holm-Swarm tumor was purified following previously used protocols[11] or was obtained commercially (Medac, Hamburg, Federal Republic of Germany). Laminin also was purified from human placenta after extraction with physiologic buffer containing EDTA.[20] Microsomes were prepared from pig kidney.[21] Various monosaccharides and oligosaccharides were obtained from commercial sources (Sigma, St Louis; Biocarb AB, Lund, Sweden).

Immunofluorescence Test. The indirect immunofluorescence test was performed as described by Mackay and Ritts[22] on cryostat sections of rat kidney, heart, and stomach, and of human heart, liver, and thyroid. Serum samples were diluted 1:10. A few tests were done with fluorescein conjugated anti-human C3 for detecting complement-fixing antibodies.

ELISA and Radioimmunoassay. The ELISA followed standard methods.[23] Microtiter plates (Immunoplates, Nunc, Wiesbaden, Federal Republic of Germany) were coated with laminin or microsomes from pig kidney at a concentration of 10 μg/mL. Serum samples were added at a dilution of 1:1,000, followed by peroxidase-conjugated monospecific anti-human IgG and IgM antibodies from goat (Medac, Hamburg, Federal Republic of Germany) at a dilution of 1:2,000. As substrate, *o*-phenylenediamine was added at a concentration of 5 mg/mL diluted in 0.2 *M* citrate buffer (pH 5.0) containing 0.01% H_2O_2. The reaction was stopped after 20 minutes by use of H_2SO_4 (25%) and measured in a Micro ELISA Autoreader (Dynatech, Denkendorf, Federal Republic of Germany) at a wavelength of 495 nm. All data are presented as optical densities (ODs). High laminin binding was defined as readings exceeding the normal average plus three standard deviations (OD = 0.3). The intra-assay and interassay variabilities were in the ranges 5% to 15% and 10% to 20%, respectively.

Laminin was labelled with iodine 125 by the chloramine T procedure and used at a fixed concentration (2 ng/0.4 mL) in radioimmunobinding and inhibition assays.[24] The latter assays followed sequential saturation protocols. Immune complexes were precipitated by goat antiserum against Cohn fraction II proteins.[16]

Immunoblot. Samples (100 μg) used for immunoblotting were separated by SDS-polyacrylamide gel electrophoresis under reducing conditions and then transferred to nitrocellulose.[25] Blots were incubated with patient sera (diluted 1:100) followed by peroxidase-conjugated polyvalent anti-human immunoglobulin serum (Dakopatts, Hamburg, Federal Republic of Germany) diluted 1:200. Bound antibodies were visualized by use of 3-amino-9-ethylcarbazole (0.4 mg/mL) as substrate, and the reaction was stopped with 7% acetic acid.

Absorption Studies. Undiluted sera were incubated with microsomes at a concentration of 30 mg/mL serum as well as with 0.01 *M* Tris-saccharose-EDTA buffer (pH 7.4) as control for 24 hours at 4°C. After centrifugation at 100,000 times gravity to remove the microsomes, the absorption was repeated twice. Immunoadsorption tests on mouse laminin coupled to agarose followed standard procedures.[24] Bound antibodies were eluted from the column with 0.2 *M* α-methyl galactose.

Statistical Analysis. Statistical analysis was performed by use of Student's *t* test. Differences with $p < .05$ were considered statistically significant.

RESULTS

Frequency of Antilaminin Antibodies. Sera from 413 patients with inner ear diseases were tested by ELISA for their reaction with mouse laminin and compared to a panel of 100 normal serum samples. Fifty-nine percent of all patients possessed high levels of these antibodies with OD readings in the range 0.3 to 1.5 (mean, 0.83 ± 0.45). Such enhanced antibody reactions (Table 1) were found particularly in patients with sensorineural hearing loss (68%), tinnitus (60%), and sudden deafness (46%), but not in patients with Meniere's disease (14%). Antilaminin antibodies (ALAs) of the IgG type predominated (Table 1).

Only 8% of normal individuals showed elevated ALA titers by ELISA (mean OD, 0.38 ± 0.13). Most of the normal serum samples, however, showed a low OD (mean 0.07 ± 0.08; similar for both IgG

TABLE 1. FREQUENCY OF ANTIBODIES AGAINST LAMININ TESTED BY ELISA IN 413 PATIENTS

			Positive for Immunoglobulins			*Mean OD ± SD*	
Diagnosis	*No. of Patients*	*No. ALA-Positive (OD = 0.3)*	*IgG*	*IgM*	*IgG + IgM*	*IgG*	*IgM*
Inner ear disorders							
Sensorineural hearing loss	296	202 (68%)	74%	12%	14%	0.51±0.88*	0.32±0.54*
Tinnitus	20	12 (60%)	83%	0%	17%	0.63±0.49*	0.25±0.35*
Sudden deafness	48	22 (46%)	100%	0%	0%	0.47±0.42*	0.14±0.05†
Meniere's disease	49	7 (14%)	100%	0%	0%	0.34±0.39*	0.12±0.05†
Autoimmune disorders							
Autoimmune chronic active hepatitis	270	16 (6%)	44%	31%	25%	0.15±0.18†	0.16±0.11†
ANA-positive and -negative collagen diseases	250	13 (5%)	23%	31%	46%	0.15±0.12†	0.17±0.10*
Control subjects	100	8 (8%)	25%	75%	0%	0.12±0.09	0.14±0.08

Immunoglobulins detected by monospecific antibodies; no analysis for IgA antibodies.

ALA — antilaminin antibody, OD — optical density, ANA — antinuclear antibody.

*Significant as compared to normal control values ($p < .01$).

†Not significant as compared to normal control values ($p > .01$).

and IgM reactions). Thus, antibody titers in normal control subjects were significantly lower than in patients with inner ear diseases ($p < .05$). Also, in patients with autoimmune disorders no significant rise of ALA as compared to the healthy control subjects could be observed.

Relation Between Antisarcolemmal, Antimicrosomal, and Antilaminin Antibodies. All patient sera that showed high laminin binding also were examined by indirect immunofluorescence testing using various rat and human tissues as substrate (Table 2). As demonstrated previously,[7] a high proportion (51%) of these preselected patients showed distinct staining of rat muscle sarcolemma (Fig 1A) and rat liver vessels. These antisarcolemmal antibodies did not fix complement. Reactions with endothelial, nuclear, and other tissue antigens were much less frequent. No staining of the antisarcolemmal antibody–positive sera was observed with human skeletal muscle and liver.

Patients with inner ear diseases also frequently showed positive ELISA reactions with pig kidney microsomes.[6] By applying the microsomes to SDS-gel electrophoresis followed by immunoblotting, a distinct band in the position of laminin A chains could be detected with ALA-positive sera, together with two other strong bands (Fig 2).

After absorption of patient sera with microsomes from pig kidney and laminin, the serum supernatants failed to react with the laminin and microsomes in ELISA, and no longer reacted with sarcolemma of muscle tissue (Fig 1B). After absorption with microsomes, all reactions with laminin and microsomes in the immunoblot were abolished completely, whereas the serum supernatant obtained after absorption with laminin still reacted with the two additional bands with microsomes, but not with the laminin A chains (not shown). Antibodies bound to a laminin adsorbent could be displaced with α-methyl galactose and showed the same sarcolemmal staining pattern observed with the original patient serum (Fig 1A,C).

Recognition of Galα1-3Gal Epitope by Laminin-Binding Antibodies. Randomly selected patients with inner ear diseases of either high ($n = 10$) or low ($n = 5$) ELISA binding to laminin (Table 1) also were analyzed by a mouse laminin RIA[16] and showed a good correspondence by either high (28% to 44%) or low (7% to 11%) RIA binding (Fig 3A). The latter reaction was, however, still significantly above the background binding (3% to 4%) and corresponded to that usually observed for most normal sera. None of the patients showed a significant RIA reaction with human laminin (Fig 3A).

The specificity of the RIA reaction was determined by inhibition assays showing complete blocking with rather low concentrations of mouse laminin. A similarly shaped inhibition profile also was observed with the disaccharide Galα1-3Gal, demonstrating that this structure composes the major epitopes (Fig 3B). The disaccharide has, however, a lower affinity for the antibody than laminin, since it has to be used in molar concentrations some 1,000 times higher than laminin, which has a content of Galα1-3Gal structures distinctly below 1% of the total mass.[16,26]

The fine specificity of the reaction was analyzed by comparing the inhibitory capacity of various monosaccharides and oligosaccharides for laminin

TABLE 2. INCIDENCE OF TISSUE-REACTING ANTIBODIES IN PATIENTS ($N = 243$) WITH INNER EAR DISEASES SHOWING HIGH ANTIBODY TITERS AGAINST LAMININ

Indirect Immunofluorescence Test Pattern	*No. Positive*
Antisarcolemmal antibodies*	125 (51%)
Antiendothelial antibodies	23 (9%)
Antinuclear antibodies	5 (2%)
Others†	38 (16%)

*Frequent association with antibodies against sinusoids of liver in most instances.

†Antibodies against thyroid, parietal cells, mitochondria, smooth muscle.

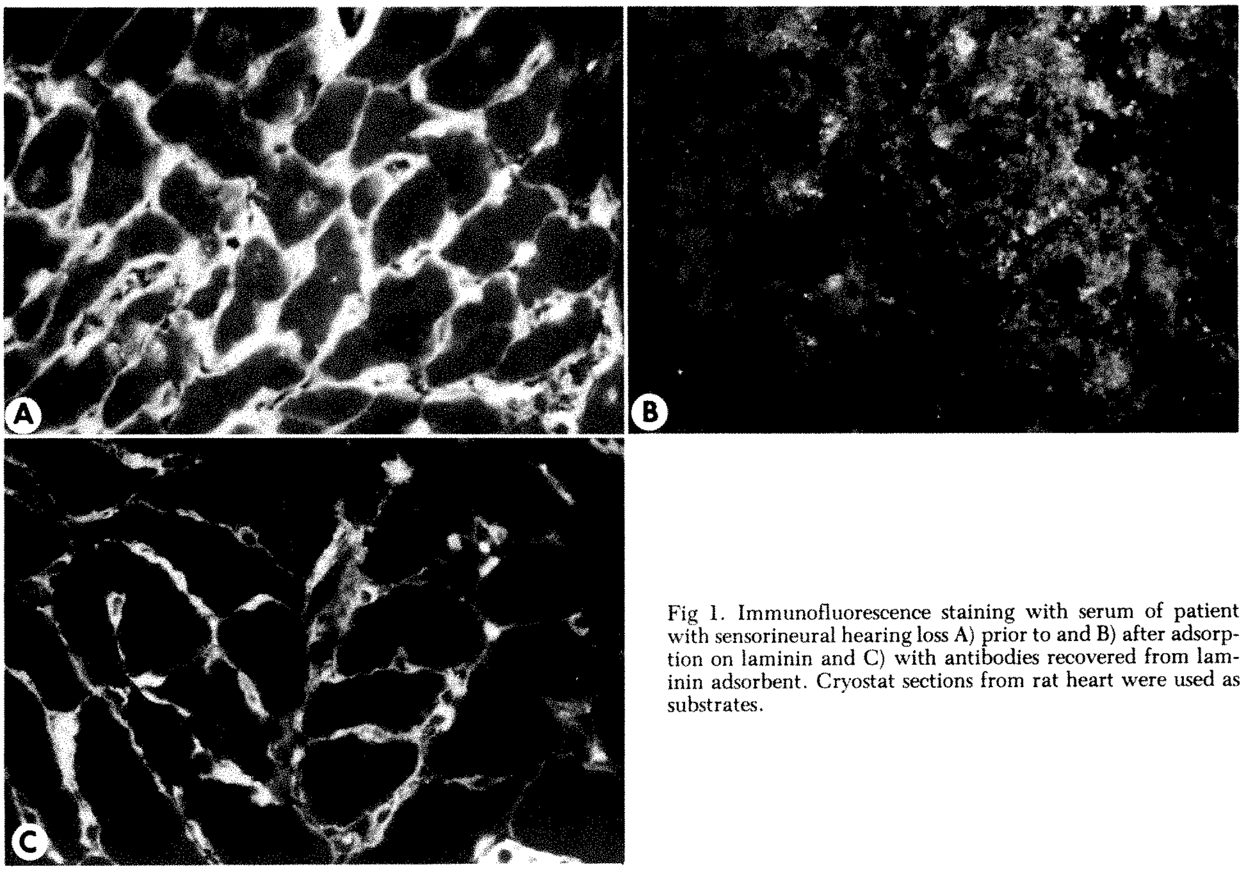

Fig 1. Immunofluorescence staining with serum of patient with sensorineural hearing loss A) prior to and B) after adsorption on laminin and C) with antibodies recovered from laminin adsorbent. Cryostat sections from rat heart were used as substrates.

binding of patient sera with high or low binding potential and of normal sera (Table 3). All three serum panels showed basically the same pattern, being more strongly inhibited by Galα1-3Gal than other galactose disaccharides or various monosaccharides. The related structure Galα1-3(Fucα1-2) Gal, which represents the B blood group epitope, also was found to be 1,000 times less efficient as an inhibitor than Galα1-3Gal (Table 3).

DISCUSSION

The ELISA data with mouse laminin demonstrated high antibody titers against the Galα1-3Gal epitope in a large proportion of patients with sensorineural hearing loss, tinnitus, and sudden deafness when compared to a normal control group. The score of strong reactions in Meniere's disease (14%) was, however, not different from those among normal individuals (8% to 15%). Carbohydrate specificity of the antibodies was assessed in inhibition assays showing the highest activity for Galα1-3Gal and a 100 to 10,000 times lower affinity for the related structures Galα1-6Gal and Galα1-4Gal. The reaction also was inhibited by galactose and α-methyl galactose but not by any other monosaccharide examined. The specificity of laminin-binding antibodies in low-titer patient sera and normal sera was similar to that in high-titer patient sera, and thus they resembled those human antigalactose antibodies examined in previous studies.[16,19]

The previously described antibodies to microsomes and muscle sarcolemma in patients with inner ear diseases[6,7] correspond to the anti-Galα1-3Gal antibodies as shown by absorption and blot experiments. This identified laminin as antigen in both reacting substances. In discriminating between high- and low-titer antibodies, however, both these assays are less sensitive and efficient than ELISA or RIA carried out with mouse laminin.

The observation that immunoblotting with microsomes showed a distinct band in the position of the laminin A chains may indicate the presence of laminin originating either from newly synthesized material in the endoplasmic reticulum or from basement material possibly contaminating such microsomal preparations.

Natural antibodies against the Galα1-3Gal epitope originally were discovered by carbohydrate affinity chromatography and rabbit erythrocyte agglutination, and are thought to represent about 1% of the immunoglobulins present in normal human serum.[18,19] Radioimmunoassay and ELISA with mouse laminin were used to determine increased amounts of these antibodies in Chagas' disease, American cutaneous leishmaniasis, and monkeys infected with *Trypanosoma*.[10,12,16] High antibody levels with Galα1-3Gal specificity were detected recently in patients with vasculitis, sarcoidosis, and infectious mononucleosis (unpublished results). Together with observations on a distinct laminin bind-

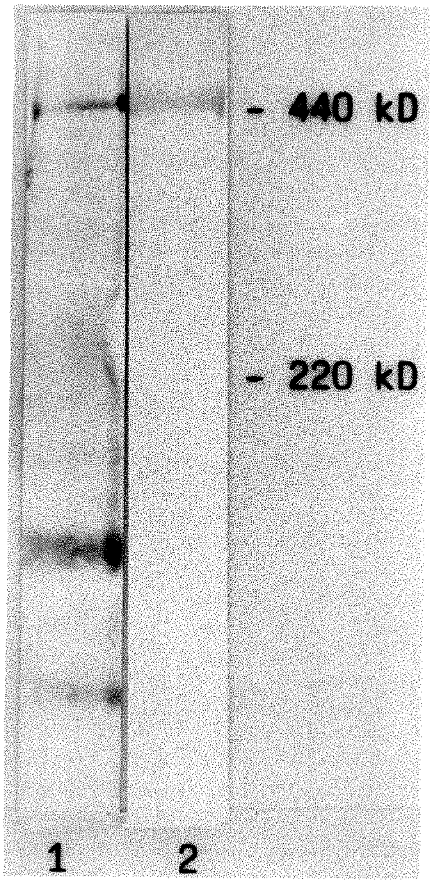

Fig 2. Comparison of pig kidney microsomes (1) with mouse laminin (2) by immunoblotting with serum from patient with sensorineural hearing loss. Serum reacts with laminin A (440 kd) but not B chains (220 kd) and recognizes same polypeptide band on pig kidney microsomes. Reactions with two additional bands were observed with microsomes that could not be eliminated by adsorption of antisera with mouse laminin.

ing in poststreptococcal glomerulonephritis[13] and preeclampsia,[14] they indicate a wide occurrence of strong antibody responses against this carbohydrate epitope in various infectious disorders and in etiologically indefinite chronic disorders.

In contrast, in sera from patients with classical non-organ-specific autoimmune disorders such as autoimmune chronic active hepatitis or collagen disorders, as well as in normal control subjects who were matched for age and sex to the patients with inner ear diseases, the incidence of this antibody type was only up to 10%. We also can say with certainty that in normal control subjects or in patients with autoimmune disorders there is no significant rise in frequency of ALA or in ALA titers in advanced stages.

So far nothing is known as to whether the antibodies are involved in protective immunity. Parasites can be responsible for triggering high responses, as demonstrated for antibodies from chagasic patients that after purification on laminin adsorbents showed distinct reactions with various forms of *Trypanosoma cruzi*.[10] This finding strongly suggests that high levels of these antibodies in patients with inner ear diseases also are caused by yet unknown infectious processes. The antibodies described here may be helpful also in identifying such infectious agents.

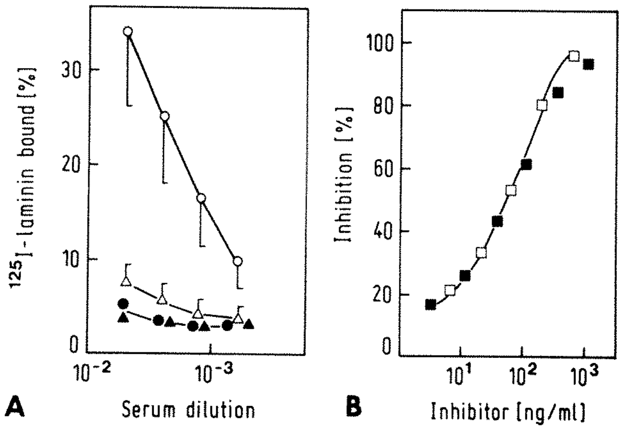

Fig 3. A) Binding of iodine 125-labelled mouse or human laminin by sera of patients with sensorineural hearing loss. Data from mouse laminin refer to average values of patients with high (n = 10; ○) or low (n = 5; △) ELISA reaction for laminin. Vertical bars indicate standard deviations. Data from human laminin were obtained from pooled sera with high (●) or low (▲) ELISA binding. B) Inhibition of binding in iodine 125-labelled mouse laminin and pooled patient serum with high binding capacity. Inhibitors used were nonlabelled mouse laminin (□) and galactosyl-(α1-3)galactose (■).

A reciprocal relationship between the presence of the Galα1-3Gal epitope and antibodies against it in the human species and Old World monkeys, compared to New World monkeys and other mammalian species that possess the epitope but lack the antibodies, implicates a strict maintenance of immune tolerance.[27] This agrees with the failure to demonstrate the epitope on human laminin. It is, therefore, unlikely that such antibodies are generated by primary autoimmune processes or because

TABLE 3. INHIBITION BY MONOSACCHARIDES AND OLIGOSACCHARIDES OF IODINE 125 LAMININ BINDING

	Concentration of Inhibitor (μM) Required for 50% Inhibition on Radioimmunoassay		
Inhibitor	*SHL +*	*SHL –*	*NS*
Galα1-3Gal	0.13	0.32	6.0
Galα1-6Gal	53	30	800
Galα1-4Gal	2,400	> 2,000	> 1,500
Galα1-3(Fucα1-2)Gal	141	250	300
α-Methyl galactose	140	1,500	500
β-Methyl galactose	8,900	10,000	>20,000
Galactose	500	11,000	>20,000
Glucose	>55,000	>55,000	>55,000
Mannose	>55,000	>55,000	>55,000
GalNAc	30,000	ND	>45,000
GlcNAc	>45,000	ND	>45,000

SHL + represents pooled sera from ten patients with sensorineural hearing loss and high titers for laminin, SHL – represents pooled sera from five patients with sensorineural hearing loss and low titers for laminin, NS — three sera (averaged) from normal subjects, ND — testing not done.

of the destruction of organs; hence inner ear diseases may be due to a secondary autoimmune reaction. It is in this context of interest that about 15% of patients with ALA-positive inner ear disease possess organ-specific antibodies against thyroglobulin or microsomes of the thyroid. This finding could indicate an activation of the immune system and the predisposition to primary and secondary autoimmune reactions in these patients.

The unexpected demonstration of high levels of carbohydrate-specific antibodies with all the possible implications discussed above may lead to a new evaluation of current concepts of the pathogenesis of inner ear diseases.[1-5] Determination of antibody levels also may have some prognostic value. Preliminary data indicate a relationship between antibody titers and disease activity, and most ALA-positive patients showed increased ESRs indicating a strong inflammatory response. There is also preliminary evidence that patients with high titers of ALA respond well to immunosuppressive therapy. A full account of these clinical observations will be given elsewhere.

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CONGENITAL ANOMALIES OF THE EUSTACHIAN TUBE IN DOWN SYNDROME

HISTOPATHOLOGIC CASE REPORT

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Congenital anomalies of the eustachian tube (ET) that may play an important role in the cause of otitis media with effusion should be investigated histopathologically. One such anomaly, which has not been thoroughly investigated to date, is that accompanying Down syndrome. This report describes histopathologic findings in a temporal bone obtained from a 26-weeks' gestation male fetus with Down syndrome. As compared to a control specimen, its ET was extremely small, mostly collapsed in the midcartilaginous, isthmus, and tympanic portions, and had poorly developed lateral cartilage in the midcartilaginous portion. This is the first case report of such ET anomalies, which appear to be due to an insult to the ET that occurs earlier in fetal life than the 16th gestational week. This insult is possibly a causative factor in postnatal ET dysfunction.

KEY WORDS — collapsed eustachian tube, congenital anomaly, histopathology, temporal bone.

INTRODUCTION

Because the mechanism by which malfunction of the eustachian tube (ET) causes otitis media with effusion is still incompletely known, it is very important to study the anatomy of the ET with its surrounding structures. To evaluate the nature of tubal malfunction, we have studied histopathologically the anomalies of the ET that may accompany conditions involving congenital anomalies. This report details the results of evaluating the temporal bones of a 26-weeks' gestation male with Down syndrome, and of comparing them with findings in the temporal bones of an age-matched normal fetus.

CASE REPORT

A 26-weeks' gestation stillborn male fetus was delivered after artificial abortion performed because Down syndrome (trisomy 21 syndrome) had been diagnosed by karyotyping of cultured amniotic fluid cells. No congenital anomalies of the internal organs were noted at autopsy, but facial anomalies such as a foreshortened forehead, low-set ears, oblique palpebral fissures, epicanthal folds, flattening of the nose, and micrognathia were present that were consistent with Down syndrome. The left temporal bone was obtained for histopathologic study.

For comparison, the left temporal bone of a grossly normal male fetus at 26 weeks' gestational age was chosen from our collection for study. The cause of death of this fetus was listed as respiratory distress due to hyaline membrane disease, and no anomalies had been noted at autopsy.

The specimens from both fetuses included not only the temporal bone but also the entire ET and its accessory structures (the ET cartilage, ET gland, tensor veli palatini muscle, levator veli palatini muscle, and hamulus).[1] After fixation in 10% formalin, decalcification in 5% trichloroacetic acid, and dehydration in graded solutions of alcohol, the specimens were embedded in celloidin and sectioned vertically in a plane perpendicular to the long axis of the petrous bone. Every tenth section of the Down syndrome specimen and every 20th section of the control specimen was stained with hematoxylin and eosin. Each of the stained sections was mounted on a clean glass slide for light microscopic study.

HISTOPATHOLOGIC FINDINGS

External and Middle Ear. The external auditory canal of the specimen from the Down syndrome fetus was normal. However, the tympanic membrane blood vessels were engorged within the connective tissue layer, and the middle ear space was developed only in the protympanum and the anterior lateral part of the mesotympanum. In addition, mesenchymal tissue filled the rest of the middle ear space. No inflammation was observed in either the middle ear space or the remaining mesenchymal tissue in the middle ear; however, a moderate number of blood vessels and a small residue of hemorrhage were seen at the epitympanum. The malleus and the incus were relatively bulky and contained a moderate amount of bone marrow. The stapes was also bulky in shape. The facial nerve, chorda tympani nerve, and Jacobson's nerve were all normal. The stapedius muscle and tensor tympani muscle were well developed in size and normal

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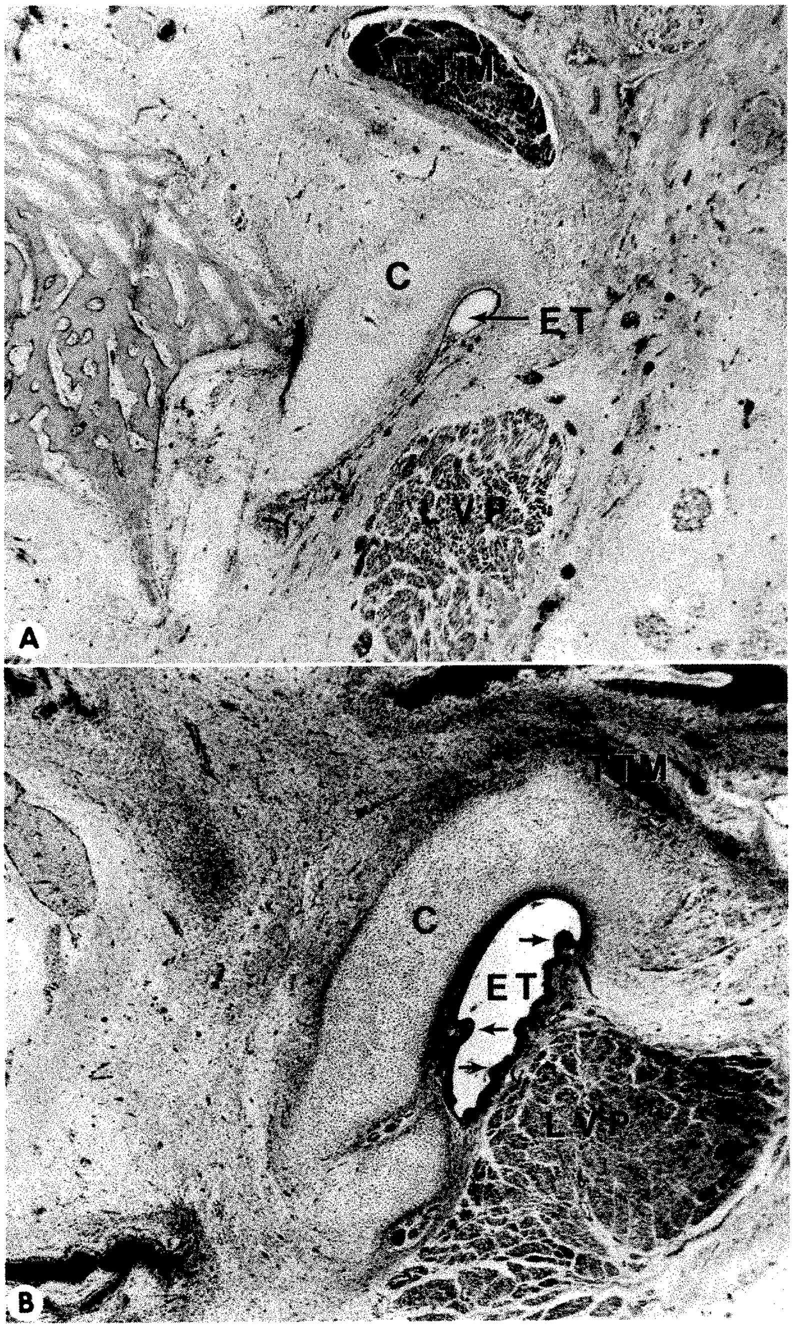

Fig 1. Isthmus portion of eustachian tube (ET) (H & E, original x29). C — tubal cartilage, LVP — levator veli palatini muscle, TTM — tensor tympani muscle. A) Down syndrome specimen. Note poorly developed ET, which has extremely narrow lumen in area just below most superior portion of tubal cartilage. B) Normal control specimen. Note well-developed ET with its lumen and epithelial infoldings (arrows).

in course. However, the facial canal and semicanal of the tensor tympani muscle were not well developed. The findings in the external and middle ear of the control specimen were similar to those in the Down syndrome specimen.

Eustachian Tube and Its Accessory Structures. In the pharyngeal portion of the ET from the Down syndrome specimen, the lumen was extremely small and narrow. Its cross-sectional length (2.51 mm) was almost the same as that of the control specimen (2.56 mm); however, its width was approximately one sixth (0.07 mm) that of the control specimen (0.42 mm). In addition, the epithelium in this portion of the ET was cuboidal and did not form a folded layer. In contrast, the epithelium of the ET of the control specimen was columnar, and did form a folded layer in its inferior half.

In the area near the midcartilaginous portion of the ET of the Down syndrome specimen, the superior half of the ET lumen was quite collapsed (cross-sectional length, 0.35 mm; width, 0.11 mm). This area was surrounded by a cuboidal epithelial layer, but the inferior half of the ET lumen was neither epithelialized nor open. In the control specimen, the ET lumen was widely open (cross-sectional length, 1.20 mm; width, 0.37 mm) and the epithelium in this area was composed of columnar cells with cilia.

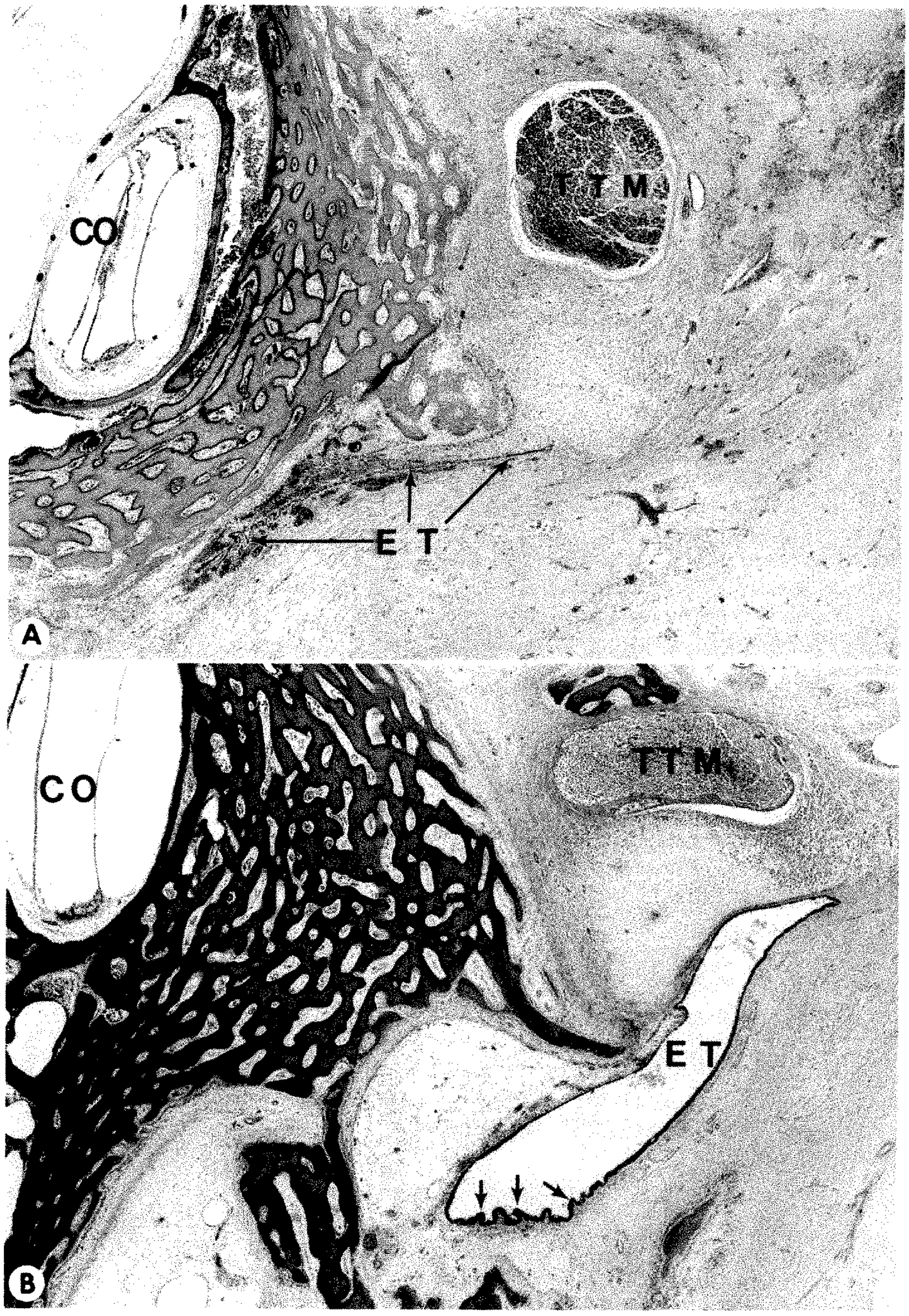

Fig 2. Tympanic portion of eustachian tube (ET) (H & E, original x34). CO — cochlea, TTM — tensor tympani muscle. A) Down syndrome specimen. Note poorly developed ET with its lumen almost completely collapsed. B) Normal control specimen. Note well-developed ET with its lumen and epithelial infoldings (arrows).

Near the isthmus portion of the ET from the Down syndrome fetus, various anomalous conditions of the ET were observed. In the most superior of the four parts of the ET the opening was small (cross-sectional length, 0.35 mm; width, 0.09 mm) and was surrounded by a cuboidal epithelial layer. The next part of the ET, moving inferiorly, was collapsed and slitlike, and the lumen was composed of remnants of a single flat epithelial cell layer. The third part of the ET evidenced neither an ET lumen nor epithelium. The most inferior part of the ET again had an extremely small lumen (cross-sectional length, 0.35 mm; width, 0.02 mm) and was composed of cuboidal epithelial cells in layers (Fig 1A).

In the isthmus portion of the control specimen the ET was well open (cross-sectional length, 1.19 mm; width, 0.36 mm). The uppermost part of the epithelium in the control specimen was composed of ciliated and low columnar epithelial cells, whereas all other parts were composed of a ciliated columnar epithelial cell layer (Fig 1B).

In the area of the Down syndrome specimen near the tympanic portion of the ET, the ET lumen was completely collapsed. However, two layers of cuboidal epithelial cells were observed side by side and spanned about 2.05 mm in cross section (Fig 2A).

In the control specimen, the ET in this area was well open (cross-sectional length, 1.98 mm; width, 0.22 mm) and the superomedial part of the ET was lined by cuboidal epithelium; however, all other parts were composed of ciliated columnar epithelium (Fig 2B).

As for the structures surrounding the ET, the tensor veli palatini muscle and the levator veli palatini muscle appeared to be normal in both the Down syndrome and control specimens. However, in the midcartilaginous portion of the ET of the Down syndrome specimen the area of the lateral lamina of the tubal cartilage was much smaller (0.08 mm^2) than in the control specimen (0.14 mm^2), although the area of the medial lamina in the Down syndrome specimen (1.90 mm^2) was slightly greater than that in the control specimen (1.58 mm^2). Mucosal gland tissue was observed in both the pathologic and normal specimens, and in neither case were many goblet cells observed. There was no evidence of inflammation in the ET or its vicinity in either the control or Down syndrome specimen.

Inner Ear. The cochlea of the fetus with Down syndrome was normally developed in all areas sampled (each 2¾ turns). The hair cells and the cochlear neurons were also well developed and were present in normal numbers. In the vestibular system, the macula utriculi was well developed and appeared to be normal, but the macula sacculi could not be evaluated because of the presence of technical artifacts. The three semicircular canals were well developed and they contained normal populations of hair cells and of vestibular neurons. These structures appeared to be normal. No other abnormality was noted in the inner ear or the internal auditory meatus of the Down syndrome specimen. The inner ear findings observed in the control specimen were quite similar to those in the Down syndrome specimen.

COMMENTS

Very few reports of congenital anomalies of the ET have been published to date. These include a defect of the ET,[2,3] a deficiency of the osseous portion of the ET,[4] a diverticulum of the ET,[2] a congenital tumor (polyp),[2] and a narrow ET lumen in its osseous part, probably due to impeded development of the tubotympanic pouch.[5] The ET abnormalities observed in the present case bore resemblances to the findings in the specimen studied by Altmann,[2] but the ET in the present case appeared to be more severely underdeveloped, especially in the area from the midcartilaginous portion to the tympanic portion, where not only was the lumen collapsed but the cross-sectional length of the ET was also less. To our knowledge, this type of ET abnormality has never been reported.

In the embryonal development of the ET, during the eighth gestational week a pair of pharyngeal pouches starts to elongate to form the auditory tubes.[6] According to Wolff,[7] in the 11th gestational week a direct communication forms between the middle ear cavity and the nasopharyngeal cavity; at this age the length of the ET is approximately 2 mm.[7] Tos[8] measured the inner circumference of the ET lumen of human fetuses of different ages, from the 12th to the 27th gestational week. The mean circumference, which represented the mean of three measurements made at the pharyngeal orifice, tympanic orifice, and middle portion of the ET, was 1.0 mm at the 12th gestational week (crown-rump length, 50 mm) and 4.4 mm at the 27th gestational week (crown-rump length, 230 mm). The circumference increased from the 12th to the 20th gestational week by about 0.15 mm per week, and from the 22nd week by about 0.30 mm per week. The mean circumference of the ET in the present case (crown-rump length, 226 mm) was extremely small (2.2 mm) for this gestational age (26 weeks); the circumference in the control specimen (crown-rump length, 270 mm) was 5.4 mm. The small circumference of the Down syndrome ET was the result of partial absence of the ET epithelium and decreased cross-sectional ET lumen in both the midcartilaginous and isthmus portions of the ET. The extremely small ET in the present case appears to be due to interruption of embryonal development of the ET earlier than 20 gestational weeks.

The columnar epithelium with cilia that lines the lumen of the normal ET first appears in the 16th gestational week.[9] A further indication that the present specimen from a fetus with Down syndrome shows arrested development is that the superior part of the ET was lined by cuboidal epithelium surrounding a small lumen, while the inferior part of the ET had neither epithelium nor a lumen in the midcartilaginous and isthmus portions. In the control specimen all parts of the ET had ciliated columnar epithelium surrounding a well-developed ET lumen in those portions. Because the Down syndrome specimen contained no evidence of inflammation of the ET that could have caused metaplasia in the epithelium of the ET, for example from the columnar cells to the cuboidal cells, the above findings in the epithelial cells of the ET appear to be the result of poor development of the ET during early gestational life.

The tubal cartilage begins to be formed at the end of the 12th gestational week, starting in the pharyngeal part of the medial lamina. By the 16th gestational week, the cartilage in the lateral lamina begins to develop, and by the 20th gestational week the tubal cartilage is well developed and has attained its final form.[8] In the present case, the tubal cartilage of the lateral lamina was still not well developed. This is further evidence that in this Down syndrome specimen the development of the ET cartilage was interrupted earlier than the 20th gestational week.

The present report is the only one known to us to date of an anomaly of the ET in Down syndrome. The ET anomalies present in this Down syndrome specimen are consistent with the notion that the otitis media that frequently occurs in these individuals[10] is partly due to malfunction of the ET by intrinsic mechanical obstruction of the ET due to a poorly developed, small ET. However, White et al[11] showed that the functional abnormalities of the ET in children with Down syndrome are a low passive tubal resistance, a low closing pressure, constriction of the ET on swallowing, and inability of most of these children to equilibrate positive or negative middle ear pressure. These factors suggest that children with Down syndrome are at high risk for otitis media with effusion because of functional obstruction of the ET. The present case therefore may be an example of an exceptionally rare ET anomaly associated with intrinsic mechanical obstruction leading to major dysfunction of the ET. In addition it appears that the tubal cartilage (lateral lamina) anomaly that presumably is associated with functional obstruction of the ET may play a role in dysfunction of the ET in this case.

Abnormalities of the middle ear in the temporal bones from patients with Down syndrome have often been observed, much more frequently than have anomalies of the inner ear.[12] The most common abnormality of the middle ear reported was mesenchymal tissue remaining in the middle ear cleft,[12,13] followed by wide angle of the facial genu,[12] and huge central marrow space of the incus and malleus.[13] As for the development of the middle ear cavity and disintegration of the mesenchymal tissue, in the 23rd gestational week the lumen of the tympanic cavity becomes wider,[6] and then in the 34th gestational week, after the mesotympanum and hypotympanum have developed, the epitympanum develops fully. In our study of normal fetuses of 26 weeks' gestation, the amount of mesenchyme remaining in the middle ear has been found to be approximately 60%. The volume of mesenchyme decreases as the fetus grows so that by 1 year after birth mesenchyme has been resorbed almost completely,[14] allowing the endoderm-lined tympanic cavity to expand.

From our study of normal 26-week fetuses, then, the amount of mesenchyme in the middle ear cavity, excluding the anterolateral part of the mesotympanum and the protympanum, in both the Down syndrome and control specimens appears to be normal for their age. However, the collapse or partial absence of the ET in the specimen from the fetus with Down syndrome makes it likely that this child would have suffered otitis media had he survived.

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EFFECT OF EYELID CLOSURE AND VOCALIZATION UPON THE VESTIBULO-OCULAR REFLEX DURING ROTATIONAL TESTING

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During rotational testing of the vestibulo-ocular reflex (VOR), eyelid closure is known to reduce both VOR gain and nystagmus frequency. We tested the effect of vocalization, such as counting aloud, on the VOR in ten healthy normal subjects. Velocity-step and sinusoidal stimuli were applied in complete darkness under three test conditions: 1) eyes open, performing mental arithmetic; 2) eyes closed, performing mental arithmetic; and 3) eyes closed and vocalizing. With velocity-step stimuli, eyelid closure significantly reduced VOR gain (peak eye velocity/head velocity) and nystagmus duration, but vocalization reversed these effects and resulted in the highest gain values. Similar results were obtained for VOR gain during sinusoidal stimulation. This study demonstrates that when eyelid closure is necessary or unavoidable during VOR testing, continuous vocalization ensures responses that are comparable to or better than those with eyes open in darkness.

KEY WORDS — eye closure, rotational testing, vestibulo-ocular reflex.

INTRODUCTION

The vestibulo-ocular reflex (VOR) produces compensatory rotations of the eyes during perturbations of the head, so that gaze remains steady. The VOR is conventionally evaluated by measuring the gain (eye velocity/head velocity) and time constant (TC) in response to sinusoidal or velocity-step (impulsive) rotational stimulation. To exclude the effects of visual following, VOR testing is performed in darkness, but sometimes this test condition is not possible and eye movements are recorded with the eyelids shut. Eye closure, however, is reported to reduce nystagmus frequency and VOR gain.[1]

In studies of the VOR in normal neonates,[2] we noted that VOR gain and TC appeared to increase when the infant was crying, even if the eyelids were closed; the response was better than that elicited from an alert, quiet infant whose eyes were open. Therefore, we set out to determine if vocalization could reverse the effects of eyelid closure in adult subjects. Preliminary results have been reported previously.[3]

SUBJECTS AND METHODS

Ten healthy subjects (six men and four women) without any history of neurologic disease or vestibular deficit participated in the study. Subjects' ages ranged from 21 to 50 years. All gave informed consent.

Horizontal eye movements were recorded by use of monocular, DC electro-oculography (EOG), and blinks were monitored with vertical EOG electrodes. A Contraves motor-driven vestibular chair was used to administer rotational stimuli. Data were recorded on an FM tape recorder and displayed on a Sensormedics chart recorder (system bandwidth, 0 to 100 Hz). Each subject was dark-adapted for approximately 15 minutes prior to the application of test stimuli. A head restraint was used to keep the subject's head fixed. Eye position was calibrated by use of ± 10° step displacements of a laser spot target at frequent intervals during the test session.

Test stimuli, for each subject, consisted of 1) two clockwise and two counterclockwise velocity steps of 76° per second for 60 seconds and 2) five sine wave cycles at 0.05 Hz and ten cycles at 0.5 Hz with peak velocities of 60°/s and 31°/s, respectively. Each test stimulus was applied under the following conditions: 1) eyes open and subject performing mental arithmetic, 2) eyes gently closed and subject performing mental arithmetic, and 3) eyes gently closed and the subject vocalizing (reciting the alphabet or counting aloud). The order of the test stimuli was the same for each subject. Test time was restricted to less than 35 minutes.

Gain was calculated as the ratio of peak slow-phase eye velocity to peak chair velocity. Slow-phase eye velocity was computed by hand. For velocity-step stimuli, peak slow-phase eye velocity was calculated from the mean of the three fastest slow phases for each response. The VOR TC was calculated for velocity-step responses by identifying the time at which the slow-phase velocity was 63% less than the peak slow-phase velocity. For the sine wave tests, at least five measurements of peak slow-

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SUMMARY OF TEST RESULTS

	Eyes Open	*Eyes Closed*	*Eyes Closed and Vocalizing*	*F*
Velocity-step				
Gain	0.67±0.15	0.46±0.13	0.86±0.17	56.33*
Time constant	17.8 ±5.20	17.40±5.40	20.80±5.60	4.39†
Nystagmus duration	34.2 ±5.10	21.50±5.30	30.60±7.90	23.78*
Sinusoidal				
0.05 Hz; gain	0.71±0.17	0.57±0.11	0.88±0.27	13.47*
0.5 Hz; gain	0.89±0.27	0.67±0.22	0.87±0.29	4.27†

Results are mean±1 SD. Solid lines indicate significant difference between test conditions ($p<.05$) by Tukey Honest Significant Difference test.

*$p<.001$

†$p<.01$

phase eye velocity were used to calculate VOR gain for each test condition. Repeated analysis of variance (ANOVA) was performed.[4] The Tukey Honest Significant Difference test was applied for post hoc comparisons.[5]

RESULTS

Group results for each of the test conditions are summarized in the Table. Typical responses to velocity-step stimuli with eyes open, eyes closed, and eyes closed while vocalizing are shown in the Figure. With eye closure, in most subjects there was a delay of approximately 1 second from the onset of the velocity-step stimulus to the onset of nystagmus. The subsequent response had a lower gain, shorter duration, and fewer quick phases. These features are shown in the Figure, B. For velocity steps, the group mean gain of the VOR for the eyes-open condition was 0.67; when the eyes were closed, the gain dropped to 0.46 (see Table). However, when subjects had their eyes closed and were vocalizing, the gain rose to 0.86.

The VOR TC was not affected substantially by either eye closure or vocalization. However, nystagmus duration was reduced significantly with eye closure. With eyes open, the group mean nystagmus duration was 34.2 seconds. When subjects closed their eyes the mean duration was 21.5 seconds. Vocalization caused nystagmus duration to rise to a mean of 30.6 seconds.

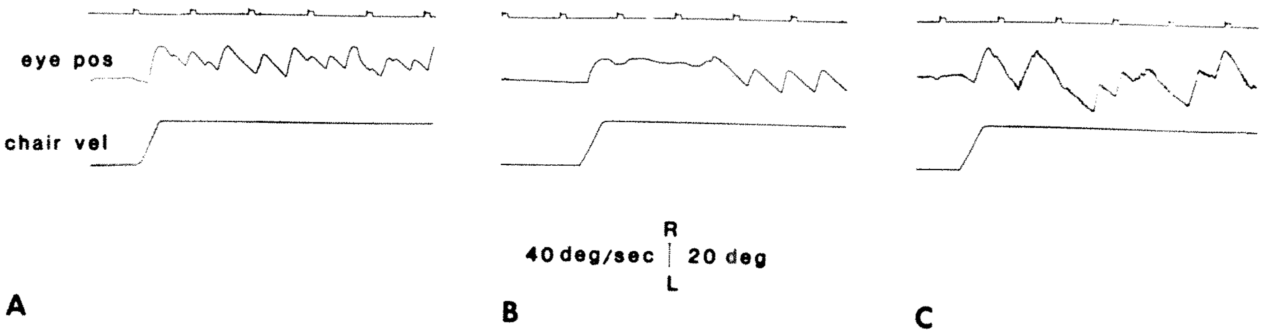
The results of eye closure and vocalization on VOR gain during sinusoidal stimulation were similar to those obtained with velocity-step stimuli (see Table). For example, with 0.5-Hz sine waves, group mean VOR gain was 0.89 with eyes open and 0.67 with eyes closed, but 0.87 with eyes closed and vocalization. Similar results were obtained with 0.05-Hz sinusoidal stimuli.

DISCUSSION

These results confirm that eyelid closure reduces VOR gain and nystagmus duration during rotational testing. Our second finding is that this effect of eyelid closure may be reversed if the subject vocalizes during testing. This effect was present with both velocity-step and sinusoidal stimuli of 0.05 and 0.5 Hz. This result complements the study of Kileny et al,[6] who demonstrated that conversation was an effective method of sustaining calorically induced nystagmus.

Tjernström[1] reported nystagmus inhibition as a consequence of eyelid closure in 20 of 30 subjects tested with rotational stimuli. Baloh et al[7] showed that caloric nystagmus often was suppressed by eyelid closure. In both of these studies, the authors attributed the suppression of vestibular nystagmus to an upward deviation of the eyes. It should be noted, however, that EOG is an unreliable method for monitoring vertical eye position because of eyelid artifact.[8] In a study using the magnetic search coil technique,[9] it was demonstrated that the most common deviation of the eyes to occur when the lids are closed is, in fact, down and inward. Since vocalization reversed the suppressive effects of eyelid closure, we doubt that vertical deviation is the cause of nystagmus suppression.

More likely, eyelid closure affected the subjects'



Typical responses to velocity-step stimuli from one subject. Time marks, at top, are in seconds. pos — position, vel — velocity. A) Eyes open, performing mental arithmetic. B) Eyes closed, performing mental arithmetic. C) Eyes closed, counting aloud. Note delay to onset of nystagmus in B, and lower-velocity slow phases; these effects are reversed by vocalization (C).

level of arousal. Impaired alertness is known to affect several aspects of ocular motor performance. For example, Henn et al[10] showed, in monkeys, that brain stem burst and pause neurons, which are important in the generation of saccades and quick phases, changed their discharge patterns with the monkeys' level of alertness. One consistent finding in our study was a reduction of nystagmus duration following the onset of the velocity-step stimulus even though the VOR TC remained unchanged. In addition, eyelid closure often led to delayed onset of nystagmus with velocity-step stimuli. Thus, reduction of nystagmus might, in part, reflect a change in the properties of the "quick-phase generator." However, lid closure also affected VOR slow phases, for example during sinusoidal stimulation, when quick-phase frequency was unchanged. Thus, eyelid closure probably exerts independent, suppressive effects on quick and slow phases of nystagmus. In contrast, the VOR TC was essentially unchanged by eyelid closure.

Conventionally, when subjects are tested in darkness with their eyes open, alertness is maintained by asking them to perform mental arithmetic.[11] Our results indicate that vocalization is equal to or better than mental arithmetic for maintaining the vestibular response. Thus, if there is concern that the subject is not attending to the mental task, he can be asked to count aloud or recite the alphabet. Engaging the patient in a conversation might also be effective.[6] We deliberately tested the effects of vocalization at the end of each session when the effects of fatigue and habituation would be maximized; nevertheless, the responses were better than those obtained earlier on. It should be noted, however, that a variety of factors other than alertness, such as spectacle refraction[12] and a mental percept of a stationary target,[13] may influence VOR gain, and these factors also should be controlled.

In some laboratories, it is not possible to test in total darkness, either for technical reasons or because of patient concerns (eg, claustrophobia). In these situations, continuous vocalization with the eyelids closed should ensure responses that are comparable to or better than those with the eyes open in darkness.

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EARTH HORIZONTAL AXIS ROTATIONAL RESPONSES IN PATIENTS WITH UNILATERAL PERIPHERAL VESTIBULAR DEFICITS

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We evaluated the vestibulo-ocular reflex (VOR) of five patients with surgically confirmed unilateral peripheral vestibular lesions. Testing used both earth vertical axis (EVA) and earth horizontal axis (EHA) yaw rotation. Results indicated that the patients had short VOR time constants, asymmetric responses to both EVA and EHA rotation, and normal EHA modulation components. These findings suggest that unilateral peripheral vestibular loss causes a shortened VOR time constant even with the addition of dynamic otolithic stimulation and causes an asymmetry in semicircular canal-ocular reflexes and one aspect of otolith-ocular reflexes.

KEY WORDS — earth horizontal axis, earth vertical axis, rotational response, vestibule, vestibulo-ocular reflex.

INTRODUCTION

The effect of surgically confirmed unilateral vestibular loss on the vestibulo-ocular reflex (VOR) of humans has been studied previously with earth vertical axis (EVA) yaw rotation,[1-3] a test of horizontal semicircular canal function. Unilateral peripheral vestibular loss has been shown to cause a short horizontal VOR time constant (increased low frequency phase lead) and a persistent response asymmetry, ie, a directional preponderance (DP).

Thus far, there has been no systematic investigation of the effect of unilateral peripheral vestibular loss on the eye movement response to earth horizontal axis (EHA) yaw rotation, a dynamic otolith stimulus. In order to assess the influence of chronic asymmetric vestibular function on otolith-ocular reflexes, we tested five patients with surgically confirmed unilateral peripheral vestibular lesions with both EVA and EHA rotation.

METHODS

Patient Population. The patient population consisted of five individuals between the ages of 45 and 67 (mean, 55.8), each of whom had a complete unilateral peripheral vestibular lesion that was confirmed surgically. The patients' ages, surgical procedures, and time of testing are listed in Table 1. Aside from their otovestibular complaints, the patients were free of neurologic signs and symptoms at the time of testing except for a diminished right corneal reflex in patient 5. Also, preoperative computed tomographic scans of each of the patients revealed no intraparenchymal CNS lesions and, for the patients with tumors, no evidence of brain stem compression.

TABLE 1. UNILATERAL PERIPHERAL VESTIBULAR LOSS PATIENT SUMMARY

Patient	*Age at Surgery (yr)*	*Sex*	*Surgical Procedure*	*No. of Months Following Surgery When Tested*	
				EVA	*EHA*
1	45	F	Left vestibular nerve section	12	4
2	51	F	Left labyrinthectomy	13	4
3	55	F	Left vestibular nerve section	18	24
4	61	F	Left acoustic neuroma resection	6	2
5	67	M	Right acoustic neuroma resection	6	2

EVA — earth vertical axis, EHA — earth horizontal axis.

Control Population. The control population consisted of five persons between the ages of 43 and 66 (mean, 55.8) who had no history of otologic or neurologic disease. These individuals had normal ocular motor, posturographic,[4] and EVA rotational responses according to normative values for the laboratory.

Ocular Motor Testing. With use of bitemporal electro-oculography (EOG), each patient underwent testing of horizontal voluntary saccades, ocular pursuit tracking, and optokinetic nystagmus. The EOG signal was fed to a DC coupled signal amplifier with a high frequency cutoff of 40 Hz. The amplified, filtered eye movements were recorded on a chart recorder for hand analysis. Saccades were tested by instructing the subjects to fixate targets placed ±10° horizontally. Pursuit tracking was tested with a target moving sinusoidally at a fre-

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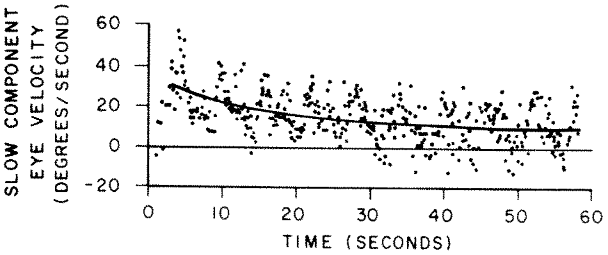

Fig 1. Horizontal slow component eye velocity versus time in normal subject during counterclockwise earth horizontal axis rotation.

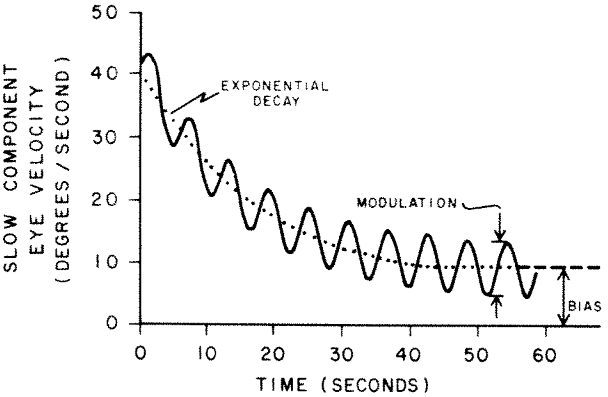

Fig 2. Diagram of slow component eye velocity response to earth horizontal axis rotation. Three response components — exponential decay, modulation component, and bias component — are indicated.

quency of 0.18 Hz and ± 12°/s. Optokinetic nystagmus was tested with projected black and white stripes that subtended about 15° per cycle. The optokinetic stimulus spanned a total of about 120° of visual arc horizontally and about 30° vertically. The stripes were rotated at a constant velocity of 22°/s and induced circularvection in normal subjects. In addition, a search was made for spontaneous nystagmus in the light, in the dark, and with eyes closed. The effect of 30° of horizontal gaze deviation was also recorded.

EVA Rotational Testing. The EVA rotational testing was performed with a conventional rotatory chair. All testing was performed with eyes open behind darkened goggles with use of EOG. Rotational stimulation consisted of both single frequency sinusoids and velocity trapezoids. Single frequency sinusoids included at least four cycles of rotation at 0.01 and 0.05 Hz at 50°/s peak velocity. Velocity trapezoids were performed with a constant acceleration of 1.5°/s/s for 60 seconds followed by a constant velocity of 90°/s for 60 seconds followed by a deceleration at 100°/s/s to stop the rotation.

The EOG signal was fed to a DC coupled signal amplifier with a high frequency cutoff of 40 Hz. The amplified, filtered eye movements were recorded on a chart recorder so that slow component eye velocity (SCV) could be computed. The SCV versus time data for sinusoidal stimulation were used to compute gain, phase, and DP of the horizontal VOR. Gain was computed as the ratio of the peak SCV to the peak rotational chair velocity. Phase was computed by first identifying both the positive-going and the negative-going zero velocity points in the nystagmus record for each sinusoidal cycle. Then, the time delay between these zeroes of eye velocity and the times at which the rotational stimulus reached zero velocity were determined. The time delays were then averaged from at least three cycles. The average time delay was then divided by the period of the stimulus (100 seconds for 0.01 Hz and 20 seconds for 0.05 Hz) and multiplied by 360 to express phase in degrees. This method of computing phase yields an estimate that is accurate if the SCV response is made up of the sum of a symmetric sinusoid and a constant DC offset. For responses that are made up of asymmetric sinusoids, this method provides a first approximation of the phase of the linear portion of the response. The DP was computed by dividing the difference between the peak right-going and the peak left-going SCV by the sum of the peak right-going and left-going SCVs.[2,5]

The SCV versus time data for trapezoidal rotations were used to compute the time constant of the horizontal VOR by plotting the natural logarithm of the SCV versus time and then measuring the slope of a regression line fit through these data.

EHA Rotational Testing. The EHA rotations used velocity trapezoids described in detail elsewhere.[6] Briefly, the patients, with eyes open behind darkened goggles, were rotated about their rostral-caudal axis while strapped to a litter. Following 0.6 seconds of rotational acceleration at 100°/s/s, the patients were maintained at a constant rotational velocity of 60°/s for 60 seconds and then decelerated to a stop. For each EHA test, the subjects were first rotated clockwise (CW), ie, from supine to right lateral to prone to left lateral to supine, for 60 seconds. Following an interval of about 5 minutes, the subjects were rotated counterclockwise (CCW).

The eye movement response to EHA rotation was recorded with EOG in the same manner as for EVA testing except that the amplified, filtered eye position signal was fed to both a chart recorder and a PDP 11/04 computer for analog to digital conversion and off-line analysis. The eye position data were analyzed by a nystagmus algorithm[7] that yielded SCV versus time data. A typical SCV versus time trace derived from the EHA responses of one of our normal subjects is shown in Fig 1. These SCV data were used to estimate three parameters: the VOR time constant (the reciprocal of the rate of exponential decay), the modulation component, and the bias component. These three response components are shown diagrammatically in Fig 2. The VOR time constant and the bias component were estimated by fitting an equation of the form

TABLE 2. EARTH VERTICAL AXIS ROTATIONAL RESPONSES

Population	*Side of Lesion*	*Phase re Velocity at 0.01 Hz (°)*	*DP at 0.01 Hz*	*Phase re Velocity at 0.05 Hz (°)*	*DP at 0.05 Hz*	*Time Constant for Trapezoids (s)*
Patient						
1	L	57.8	26.7%	20.4	11.2%	5.7
2	L	72.8	27.1%	31.3	25.5%	5.5
3	L	67.1	24.0%	26.1	5.6%	7.2
4	L	74.9	10.8%	29.7	13.6%	7.0
5	R	77.1	−12.9%	23.9	−12.1%	6.2
Controls (mean ± 2 SD)		45.0 ± 12.6	7.2% ± 4.8%	12.0 ± 11.6	2.8% ± 2.6%	12.0 ± 5.2

DP — directional preponderance.

$SCV(t) = Ae^{-t/T} + B$ through the SCV data, where A is the initial value of the exponential decay, T is the time constant of the decay of response, and B is the bias. Following the subtraction of this exponential decay plus nonzero bias from the SCV data, the modulation component was estimated by least squares fitting a sinusoid of 0.167 Hz through the residual SCV data. Harmonic distortion was assessed by fitting sinusoids of multiples of 0.167 Hz through these same data.

In addition to estimating the value of the three EHA response parameters mentioned above, an asymmetry measure comparable to DP was computed for the EHA responses. This was accomplished by dividing the difference of the magnitudes of the bias components by their sum, ie, CW bias minus CCW bias divided by CW bias plus CCW bias. For those subjects in whom the sum of the absolute values of their bias components did not exceed a total of 5°/s, no DP value was reported, because small absolute values can lead to erroneously large asymmetry measures if SCV values are corrupted by a small amount of noise. When the bias component had the same sign for both directions of rotation, we assigned a value of ±100% DP.

RESULTS

Ocular motor testing revealed that each patient had normal saccades, pursuit, and optokinetic nystagmus. No patient had a spontaneous nystagmus, either with or without fixation, and no patient had gaze-evoked nystagmus.

The results of EVA rotational testing are shown in Table 2. It can be seen that the magnitude of the DP at 0.01 and 0.05 Hz was outside normal limits (defined as the mean ± two standard deviations) in nine of ten trials and that the sign of the DP coincided with the side of lesion (ie, a negative DP with right-sided lesions and vice versa) in ten of ten trials. The phase lead at 0.01 Hz was greater than normal for each patient, and the VOR time constant was shorter than normal for three of the patients. There was no obvious correlation between the magnitude of the DP and the magnitude of the time constant estimates.

A representative SCV versus time trace for a patient response to EHA rotation is shown in Fig 3. Note the shortened VOR time constant and a larger bias component when rotation was in the CW direction (away from the side of lesion).

Shown in Table 3 are the results of EHA rotational testing in the patient group. Note that the responses of the control group are highly variable and that as a result, most of the patients' response parameters fall within normal limits. However, it can be seen that the VOR time constant in all five cases is shorter than the average time constant of the normal group. It also can be seen that the magnitude of the DP exceeds normal in two of the four cases reported and that the sign of the DP coincides with the side of lesion in each of these four cases. The sign of the DP from EHA testing agreed with the sign of DP from EVA testing in eight of eight comparisons. As for EVA testing, there was no obvious correlation between the magnitude of the DP and the magnitude of the time constant estimates.

The magnitude and timing of the modulation component of EHA responses were within normal limits for each patient. Also, the total harmonic distortion in the modulation component of the patients' responses was not significantly different from the total harmonic distortion seen in the responses of normal subjects.

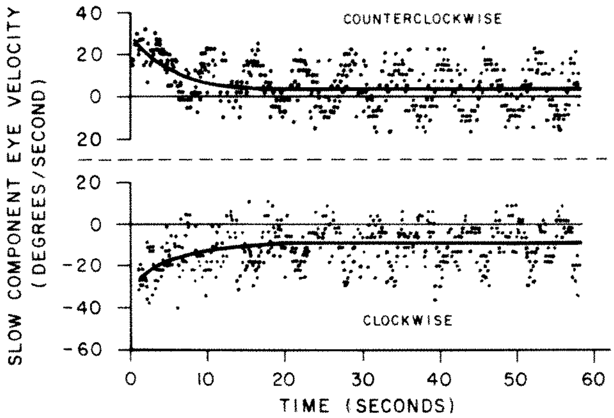

Fig 3. (Case 4) Earth horizontal axis rotational responses of patient 2 months following resection of left acoustic neuroma. Top half of figure represents counterclockwise responses, and bottom half represents clockwise responses. Note rapidity of decay of horizontal slow component eye velocity and larger bias component when rotation was toward intact side, ie, clockwise.

TABLE 3. EARTH HORIZONTAL AXIS ROTATIONAL RESPONSES

Population	Side of Lesion	Time Constant (s)	CW Bias (°/s)	CCW Bias (°/s)	DP
Patient					
1	L	3.5	−24.5	− 0.4	100.0%
2	L	2.9	−16.6	12.7	13.4%
3	L	6.2	− 9.2	1.9	66.5%
4	L	5.2	− 9.0	3.2	47.7%
5	R	8.0	− 2.1	1.0	—
Controls (mean ± 2 SD)		10.0 ± 10.2	−9.9 ± 8.2	12.9 ± 8.6	17.4% ± 37.2%

CW — clockwise, CCW — counterclockwise, DP — directional preponderance.

DISCUSSION

Unilateral peripheral vestibular loss is known to alter the eye movement response to horizontal semicircular canal stimulation using EVA yaw rotation.[1-3,8] Our study indicates that the response to EHA yaw rotation, which stimulates both the horizontal semicircular canals and the otoliths, also is altered by unilateral peripheral vestibular deficits. Specifically, both EVA and EHA testing reveal a shortened time constant of the horizontal VOR.

The pathophysiology of a shortened VOR time constant following unilateral peripheral vestibular loss is not known. It has been postulated by Baloh et al[9] that the CNS shortens the VOR time constant as a means of preserving performance at high rotational frequencies, which are most important for maintenance of gaze stability during head movement. This same mechanism may be responsible for the shortened VOR time constants seen in this study using EHA rotation.

Despite the long delay between our patients' surgical procedures and their vestibular testing, a persistent vestibulo-ocular imbalance, ie, a DP, was found in EVA rotational responses. The sign of this DP coincided with the side of vestibular loss in all test trials, ie, a left or negative DP was associated with a right-sided lesion. This correlation between the sign of the DP on EVA rotational testing and the side of vestibular loss is in agreement with the results of others.[1-3,8] Thus, a DP on EVA rotational testing could be regarded as a lateralizing sign except that occasionally the side of lesion and the sign of DP do not coincide.[1,2,8]

We also found a DP in our patients' responses to EHA rotation. As in EVA testing, the sign of this DP coincided with the side of vestibular loss in nearly all test trials. This finding is in agreement with that of Denise,[10] who evaluated six patients with unilateral peripheral vestibular deficits using off-vertical axis rotational testing (an otolith-ocular stimulus similar to EHA) and found an association between the sign of bias component asymmetry and the side of the lesion. Thus, a DP with EHA rotational testing may be a reflection of asymmetric dynamic otolith input to the CNS, and in this sense such a DP could be regarded as a lateralizing otolithic sign. However, the DP in EHA responses actually may be a nonlateralizing finding analogous to a DP in the response to EVA rotation or to bithermal caloric irrigation.[11-13] Also, it should be noted that EHA response parameters in normal subjects are quite variable, thus raising some doubt regarding the clinical usefulness of this test without further investigational studies.

The normality of our patients' modulation components, including magnitude, timing, and symmetry, suggests that the generation of this periodic component does not require bilateral otolithic input. However, the large total harmonic distortion and high degree of variability of both the magnitude and the timing of the modulation component[6] may have obscured subtle effects of unilateral otolithic loss.

Our data show that the response to EHA yaw rotation, a combined horizontal semicircular canal and dynamic otolith stimulus, is altered by unilateral peripheral vestibular loss. Further research is needed to determine the time course of such response alterations and to determine the clinical benefit that may be obtained from otolith-ocular reflex testing.

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ELECTROCOCHLEOGRAPHIC CHANGES IN ENDOLYMPHATIC HYDROPS INDUCED BY TYPE II COLLAGEN IMMUNIZATION THROUGH THE STYLOMASTOID FORAMEN

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Changes in action potential (AP) and summating potential (SP) were investigated in guinea pigs immunized with type II collagen through the stylomastoid foramen. Endolymphatic hydrops could be induced in four of 11 guinea pigs. The striking feature of the electrocochleographic waveform in guinea pigs with endolymphatic hydrops was the negative SP recording in response to high frequency tone bursts. Furthermore, abnormal changes in AP were observed in three of four hydropic guinea pigs. Morphologic study of the cochleas in these three guinea pigs with light microscopy revealed spiral ganglion cell degeneration in addition to endolymphatic hydrops and almost normal sensory hair cells. These results suggest that guinea pigs with hydrops as produced by our procedure can serve as a useful model of Meniere's disease, that autoimmune response may play an important role in the etiopathogenesis of Meniere's disease, and that spiral ganglion cell degeneration together with endolymphatic hydrops seems to contribute to abnormal changes in AP.

KEY WORDS — action potential, electrocochleography, endolymphatic hydrops, immunization, summating potential, type II collagen.

INTRODUCTION

There are many reports that abnormal changes of the summating potential (SP) are characteristically observed in electrocochleography (ECoG) obtained from patients with Meniere's disease.[1-6] Most of these reports, using wide-band clicks as acoustic stimuli, indicated a negative SP enlargement or a negative SP dominance. Presently this SP finding is applied clinically to the diagnosis of Meniere's disease, though the generating mechanism of such an abnormality in SP still remains unexplained. Recently, Dauman et al[7] showed in ECoG for Meniere's patients, using tone burst stimuli, that those who displayed large negative SP values at low frequencies also showed large positive values at high frequencies. Gibson et al,[3,4] Morrison et al,[8] and Moffat[9] have suggested that the SP enlargement observed in ECoG in Meniere's disease is closely related to endolymphatic hydrops.

Electrophysiologic investigations of the cochlear function in experimentally induced endolymphatic hydrops are very important for getting a good grasp of the pathophysiology in Meniere's disease. Several studies have been presented concerning this subject. Kumagami et al,[10] Aran et al,[11] Goin et al,[12] Prijs and Harrison,[13] Horner and Cazals,[14,15] and Harrison et al[16] have reported ECoG changes in animals with endolymphatic hydrops produced by Kimura's method.[17] Their results were not always in agreement with results of ECoG obtained from Meniere's patients. Recently, Yoo et al[18,19] reported that endolymphatic hydrops could be induced in guinea pigs by immunization with type II collagen, and they suggested the participation of an autoimmune response in the etiopathogenesis of Meniere's disease. In the present study, we also were able to induce experimental endolymphatic hydrops by injection of type II collagen through the stylomastoid foramen in guinea pigs that already had been sensitized with type II collagen. The purpose of this report is to study the changes of the SP and the action potential (AP) recorded from the round window in guinea pigs immunized by our procedure.

MATERIALS AND METHOD

Animals. Eleven healthy Hartley guinea pigs with normal Preyer's reflex, each weighing 300 to 400 g, were used for type II collagen immunization. Six normal guinea pigs were used as controls. Two guinea pigs were used for the comparative study as mentioned below.

Immunization. Eleven guinea pigs received 400 μg of native bovine type II collagen emulsified with complete Freund's adjuvant by intradermal injection into the foot pads or the back for sensitization. This injection was repeated four or five times at weekly intervals. Seven days after the last injection, they received the antigen challenge (200 μg type II collagen) through the right stylomastoid foramen. Kumagami et al[20] experimentally confirmed that various solutions injected through the stylomastoid foramen could reach the inner ear of rabbits. We

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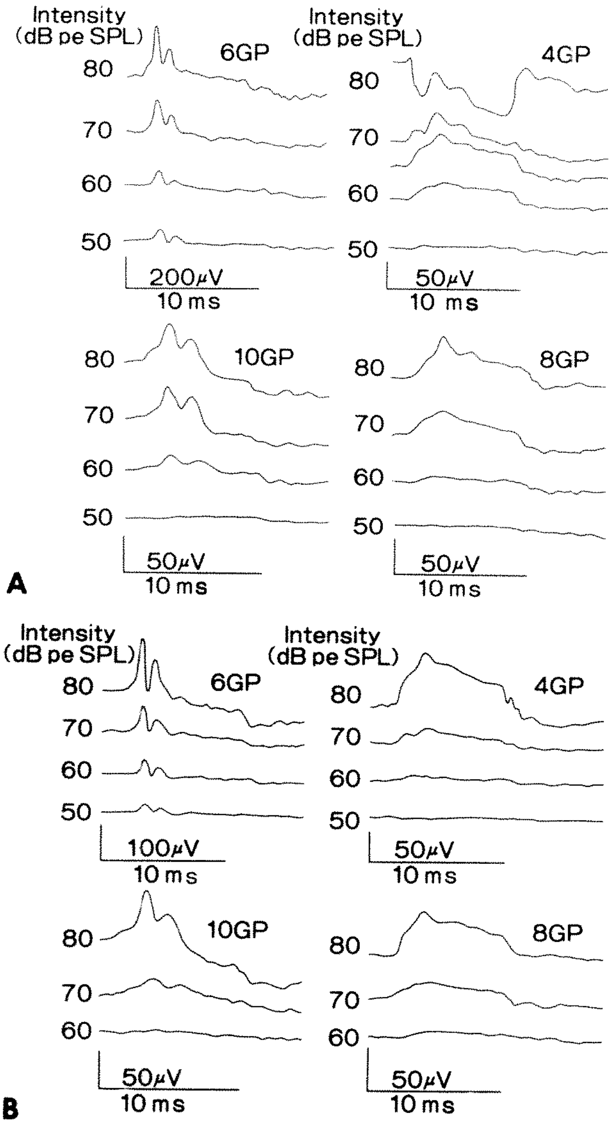

Fig 1. Electrocochleographic waveforms obtained from four guinea pigs with endolymphatic hydrops, in response to A) 8-kHz tone bursts and B) 4-kHz tone bursts.

applied this approach to directly challenge the inner ear of our guinea pigs.

The injection through the stylomastoid foramen was performed as follows. With the use of general anesthesia by intraperitoneal injection with pentobarbital sodium, a postauricular incision was made, and under an operating microscope, the facial nerve and the stylomastoid foramen were exposed. The tip of a 27-gauge needle was inserted 3 mm into the facial nerve in the stylomastoid foramen and type II collagen (200 μg) was injected slowly. An electrophysiologic measurement was done 48 hours after reception of the antigen challenge, taking into account the time course of the development of cytotoxic immune reaction. As a control, two additional guinea pigs without any previous sensitization received the antigen (200 μg) in the same way through the stylomastoid foramen, and ECoG was also measured in the same way.

Measurements. Each guinea pig underwent tracheotomy under general anesthesia with intraperitoneally administered pentobarbital sodium (Nembutal, 28 mg/kg). Guinea pigs then were given suxamethonium chloride (Relaxin, 50 mg/kg) intramuscularly and were ventilated artificially. The right tympanic bulla was exposed via the ventrolateral approach. An active electrode with a small platinum ball was placed on the round window, while a reference electrode and a ground electrode were inserted into the retroauricular muscle and the scalp, respectively. Electric responses elicited from the round window were amplified by a biophysical amplifier (DIA Medical DPA-100M) with a bandpass setting from DC to 30 kHz and a gain of 1,000x or 2,000x, and then were recorded on magnetic tape by a data recorder (TEAC R410). The signals from the data recorder were averaged and analyzed afterward by an off-line technique using a signal processor (Sanei 7T08) and a microcomputer (Tektronix 4051).

Acoustic stimuli consisted either of short tone bursts having a trapezoid envelope with a 2-cycle rise-fall time and an 8-ms plateau for frequencies of 1, 2, 4, 6, and 8 kHz, or clicks driven by a 1-cycle, 8-kHz sine wave. These stimuli were the same as those used in our routine human ECoG. Acoustic stimuli were given in an open acoustic system through a driver unit (Altec 388-16G) with an exponential horn (Altec 311-90) at a distance of 75 cm from the animal's ear entrance. Polarity of stimuli was alternated to cancel out any microphonic potentials. Sound waveforms were picked up by a condenser microphone (Rion UC-11) placed at the entrance to the external auditory meatus of guinea pigs and analyzed by a fast Fourier transformation (FFT) analyzer (Ono Sokki CF-300).

After measurements of AP and SP, guinea pigs were killed painlessly and their temporal bones were dissected en bloc and fixed with 10% neutral buffered formalin. The specimens were embedded in paraffin and cut horizontally for the morphologic studies. Serum antibody level was measured by ELISA.

RESULTS

Changes in Summating Potential. Experimental endolymphatic hydrops could be induced in four of 11 guinea pigs by immunization with type II collagen injected through the stylomastoid foramen. The ECoG from four hydropic ears showed an abnormal SP with negative polarity in response to high frequency tone bursts (4 to 8 kHz). In Fig 1A, the ECoG responses of four hydropic guinea pigs in response to 8-kHz tone bursts are shown as a function of stimulus intensity. An appearance of negative SP is characteristically exhibited. Furthermore, two guinea pigs (4GP and 8GP) showed the dominant negative SP. Although a positive SP was

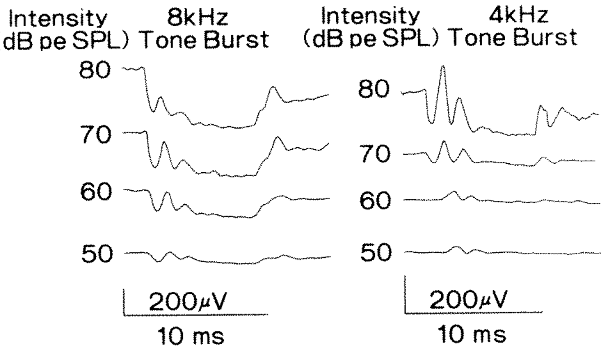

Fig 2. Response waveforms obtained from guinea pig injected with type II collagen into stylomastoid foramen without previous sensitization.

recorded in one case (4GP) at 80 dB peak equivalent sound pressure level (pe SPL), polarity change of SP from positive to negative is observed by a decrease in intensity below 70 dB pe SPL. Figure 1B indicates AP and SP in response to a 4-kHz tone burst stimulus in the same hydropic animals. Negative SP waveforms are also shown as abnormal features. Two guinea pigs that had not been sensitized previously were injected with type II collagen into the stylomastoid foramen for comparison. The ECoG waveforms obtained from one guinea pig are shown in Fig 2. The positive SP is demonstrated in response to 4-kHz and 8-kHz tone bursts; it was similar to the response of the control animals. Figure 3 depicts the comparison of SP versus frequency function curves in four hydropic guinea pigs with a control curve obtained from normal guinea pigs. The changes of SP amplitude and SP polarity are represented as a function of tone burst frequency at an intensity of 70 dB pe SPL. In the control curve, the positive SP is observed for tone bursts with a higher frequency than 4 kHz and small negative SP is seen with 1-kHz or 2-kHz tone bursts. On the other hand, in hydropic ears the negative SP is observed for tone burst stimuli above 4 kHz, but SP is hardly visible with lower frequency tone bursts.

Morphologic studies of the four hydropic cochleas showed a Reissner's membrane moderately extending toward the scala vestibuli (indicating mild endolymphatic hydrops), eosinophilic precipitates without cell infiltration in the scala media, and moderate degeneration of spiral ganglion cells that was observed in three of the four hydropic animals. No degeneration of the sensory hair cells in the organ of Corti was found with light microscopy. These pathologic changes in the cochlea were similar to those reported by Yoo et al.[19] Histologic photomicrographs indicating the representative mild endolymphatic hydrops and a moderate loss of the ganglion cell population are presented in Fig 4. In the remaining seven immunized guinea pigs, which did not develop hydrops, SP amplitude and SP polarity were similar to those in control guinea pigs. It became apparent from the above results that an unusual negative SP recording in ECoG elicited from the round window in response to high frequency tone bursts may be strongly related to the generation of endolymphatic hydrops induced by our procedure.

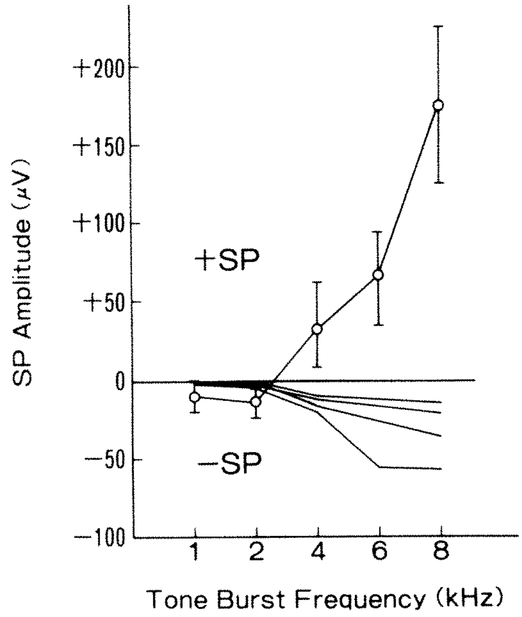

Fig 3. Summating potential versus frequency functions obtained from four guinea pigs with endolymphatic hydrops (—) and from normal guinea pigs (○—○). Vertical range bars indicate ±1 SD.

Changes in Action Potential. The changes in AP amplitude and AP latency to click stimuli were investigated in 11 immunized guinea pigs as a function of intensity. The AP input-output curves and AP latency-intensity function curves from the four guinea pigs with hydrops are depicted in Fig 5A,B. One animal (6GP) exhibits a normal input-output curve and a normal latency-intensity function curve, while the other three reveal threshold elevations (30 to 40 dB normal hearing level [nHL]), a decrease in amplitude, recruitment in the input-output curves (Fig 5A), and furthermore the prolongation of latency (Fig 5B). It was interesting to note that the guinea pig from which normal AP responses were recorded showed an almost normal spiral ganglion, and the three guinea pigs that showed abnormal AP responses had moderate degeneration of spiral ganglion cells.

Of the remaining seven guinea pigs, which did not develop hydrops, four show normal input-output curves while three, in whose cochleas a 30% to 40% loss of spiral ganglion cells was observed, show slight abnormal changes in AP such as threshold elevations (15 to 30 dB nHL), a decrease in amplitude especially at intensities below 60 dB nHL, and recruitment in input-output curves (Fig 5C). In latency-intensity function curves (Fig 5D), the prolongation of latency is not seen in any of the seven animals.

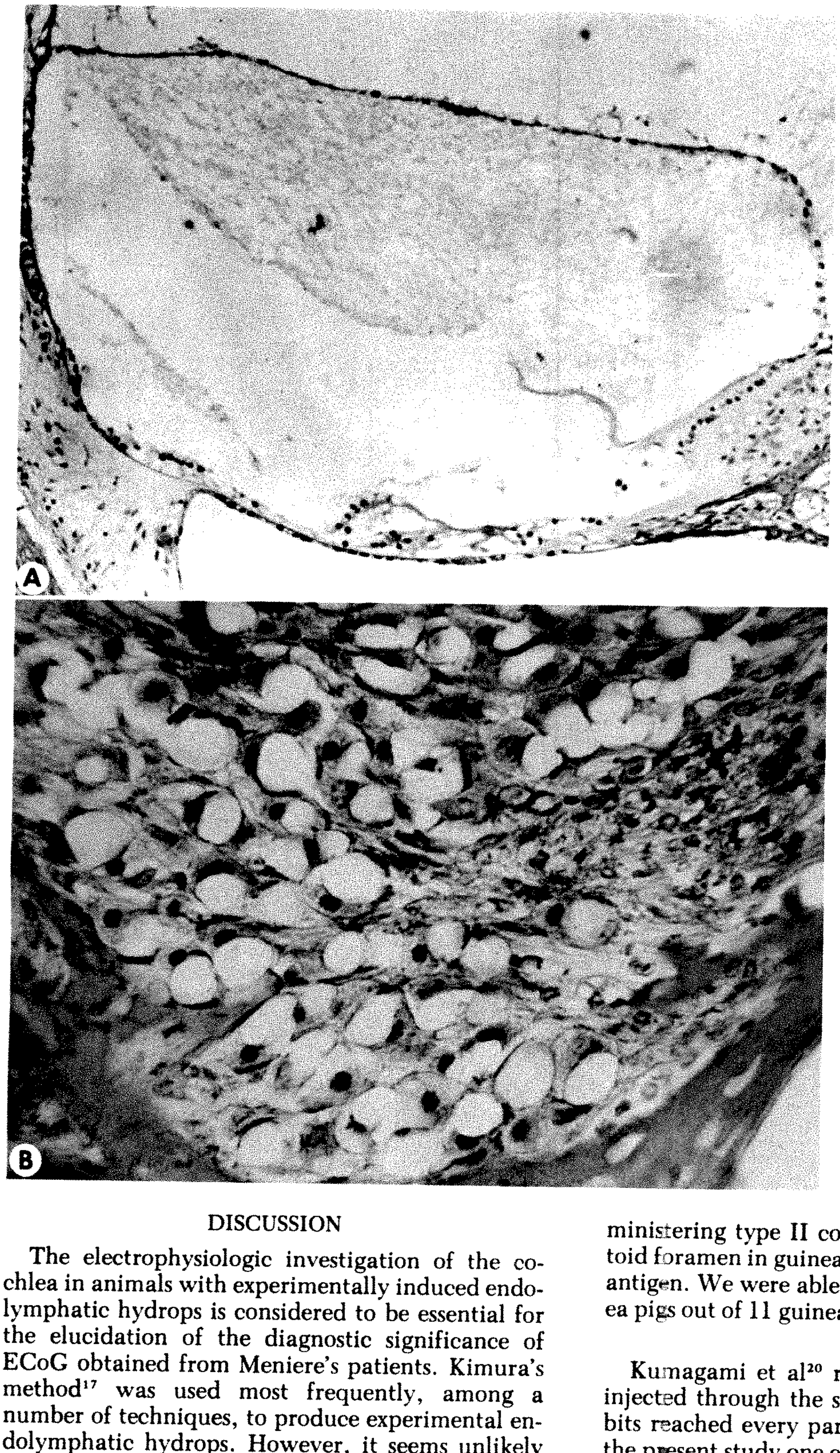

Fig 4. Photomicrographs from cochleas of guinea pigs exposed to type II collagen immunization through stylomastoid foramen. A) Mild endolymphatic hydrops (H & E, original x200). B) Spiral ganglion cell degeneration (H & E, original x400).

DISCUSSION

The electrophysiologic investigation of the cochlea in animals with experimentally induced endolymphatic hydrops is considered to be essential for the elucidation of the diagnostic significance of ECoG obtained from Meniere's patients. Kimura's method[17] was used most frequently, among a number of techniques, to produce experimental endolymphatic hydrops. However, it seems unlikely for the etiopathogenesis of Meniere's disease that the endolymphatic duct is occluded abruptly and mechanically. On the contrary, the immunologic method of Yoo et al[18,19] is thought to be a natural, ingenious procedure in this respect. We considered that endolymphatic hydrops could be caused by administering type II collagen through the stylomastoid foramen in guinea pigs sensitized with the same antigen. We were able to obtain four hydropic guinea pigs out of 11 guinea pigs immunized in this way.

Kumagami et al[20] reported that any substances injected through the stylomastoid foramen in rabbits reached every part of the inner ear. But from the present study one cannot determine the immune site in the inner ear or the pathogenic mechanisms in the development of endolymphatic hydrops. In four hydropic ears the negative SP was recorded characteristically in response to high frequency tone bursts (4 to 8 kHz). The SP elicited from the round window in response to high frequency tone bursts

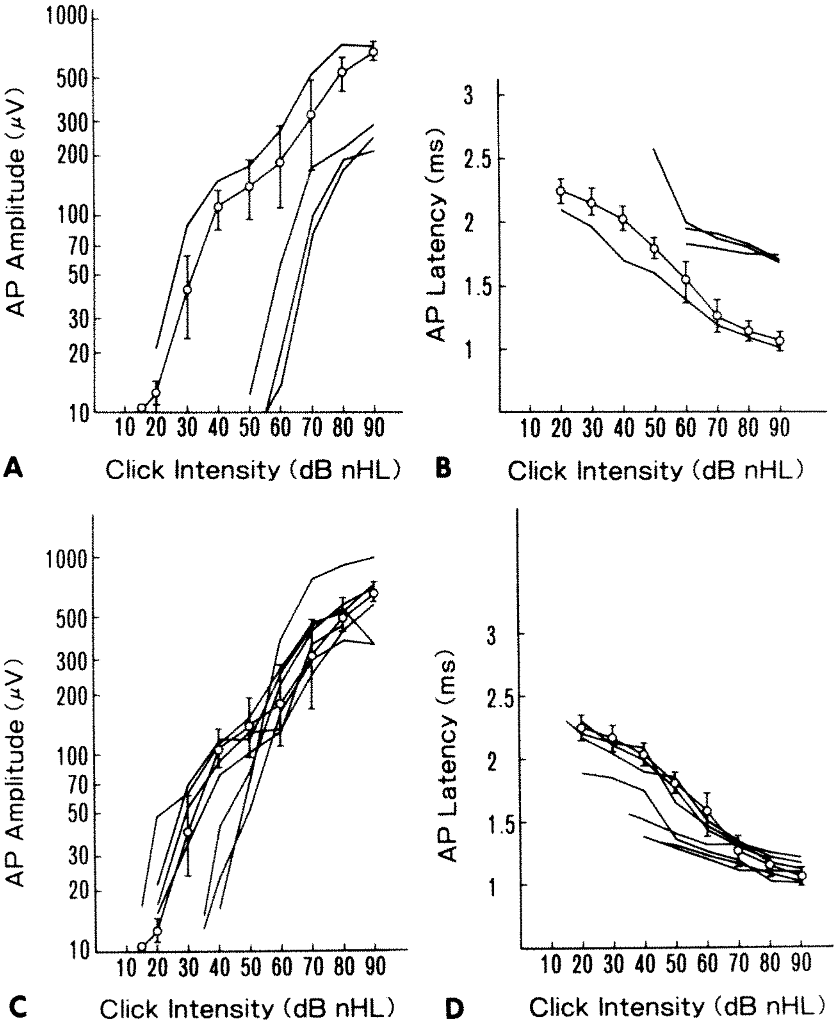

Fig 5. A) Action potential (AP) input-output curves obtained from four guinea pigs with endolymphatic hydrops (—) and from control animal (○—○). B) AP latency-intensity function curves obtained from four hydropic guinea pigs (—) and from control animal (○—○). C) AP input-output curves obtained from seven guinea pigs that did not develop endolymphatic hydrops (—) and from control animal (○—○). D) AP latency-intensity function curves from these seven guinea pigs (—) and from control animal (○—○). Vertical range bars indicate ±1 SD.

shows a positive SP polarity. Therefore the reversal of SP polarity to negative caused by the generation of endolymphatic hydrops suggests that some pathologic process in the inner ear has occurred that causes the negative SP component to become dominant. Such a negative SP dominance does not seem to be caused by damage to hair cells, because a normal population of hair cells was discovered with light microscopy. It might be related to the disturbances of basal membrane movement resulting from hydrops[4,9,21] or to the malfunction of hair cells caused by some pathologic condition resulting from an immune response. Aran et al[11] always obtained a positive SP in response to 16-kHz tone bursts from the round window of guinea pigs with endolymphatic hydrops induced by Kimura's method,[17] and they never observed a reversal of SP polarity. The difference between their results and ours leads to the supposition that the pathophysiologic process in the cochlea induced by obstruction of the endolymphatic duct may be different from the process caused by an autoimmune response.

In ECoG from Meniere's disease, abnormal SP findings such as an enlarged negative SP and an abnormal increase in a negative SP/AP ratio have been widely seen.[1-6,8] These findings were obtained with wide-band clicks as acoustic stimuli in most of the studies. Eggermont,[1] Kanzaki et al,[22] Ohashi,[23] and Dauman et al[7] performed ECoG using tone burst stimuli in humans. They found that the polarity of SP elicited from the human promontory is frequency dependent and is influenced by the site of electrode placement.[22,23] Dauman et al[7] investigated the tone burst ECoG in Meniere's patients and demonstrated that those who displayed a large negative SP value at 2 kHz also showed a large positive value at 8 kHz. Our results[23] in Meniere's patients showed, in contrast to their results, that although a negative SP characteristically enlarged at 2 to 4 kHz, a positive SP value at 8 kHz was smaller than that obtained from normal control ears. It remains unknown in ECoG of Meniere's disease whether overall SP (both positive and negative components) enlarges, or whether the negative component becomes especially dominant.[23,24] However, at present it may be said that the negative SP dominance recorded from our hydropic guinea pigs is similar to the abnormal SP finding described in many past ECoG studies of Meniere's disease. Yoo et al[25] reported a marked elevation of antibody levels to type II collagen in patients with Meniere's disease.

As concerns changes of AP response, the following phenomena were observed in our hydropic guinea pigs: an elevation of threshold, a decrease in amplitude, a recruitment aspect in the input-output curve, and a prolongation of latency. Since the light microscopic examination showed little damage in the organ of Corti, these abnormalities in AP do not seem to result from hair cell loss in the cochlea. Although the development of endolymphatic hydrops is supposed to be chiefly responsible for these abnormalities in AP, a loss of spiral ganglion cells cannot be neglected as a cause, because the guinea pigs that did not develop hydrops but revealed spiral ganglion cell degeneration also demonstrated slight abnormal changes of AP. Aran et al,[11] Kumagami et al,[10] and Horner and Cazals[14,15] investigated the changes of AP in guinea pigs with endolymphatic hydrops induced by obstruction of the endolymphatic duct. The results of Aran et al[11] and Kumagami et al[10] were similar to ours. Horner and Cazals[14] demonstrated fluctuating raised AP thresholds and a decrease in AP latencies to tone pip stimuli at low and middle frequencies. The results of Horner and Cazals concerning AP latency were contrary to those of Kumagami et al[10] and us.[23] We recognized prolonged AP latencies in three of four hydropic guinea pigs. We suppose that the difference between our results and those of Horner and Cazals may be attributable to the difference in both acoustic stimuli and cochlear damage. The AP findings obtained from our hydropic guinea pigs have a resemblance to those from Meniere's patients.[8,23,26,27] As for AP latency in Meniere's disease, considerable variation has been reported in past studies.[8,26,27]

In conclusion, from the foregoing descriptions it may be said that ECoG recorded from our hydropic guinea pigs is largely if not entirely similar to ECoG reported in past studies of Meniere's disease. Thus, although the number of experimental animals was limited, we believe that guinea pigs with immunologically induced endolymphatic hydrops can serve as a useful model of Meniere's disease, not only in its morphologic but also in its ECoG aspects. Furthermore, we support the notion that the autoimmune response may play an important role in the pathogenesis of Meniere's disease.

SUMMARY

The changes of AP and SP were investigated in 11 guinea pigs immunized with type II collagen through the stylomastoid foramen. Endolymphatic hydrops could be produced in four of 11 immunized guinea pigs. In the hydropic ears a characteristic SP finding, that is, a negative SP recording in response to high frequency tone bursts, was observed. In the remaining seven guinea pigs, which did not show the development of hydrops, SP polarity and SP amplitude were similar to those of control animals. As regards AP, three of the four hydropic guinea pigs showed abnormal changes of AP, such as a threshold elevation, a decrease in amplitude, a recruitment aspect in the input-output curve, and a prolongation of latency. Morphologic studies of the cochleas of these three guinea pigs with light microscopy revealed the degeneration of spiral ganglion cells in addition to endolymphatic hydrops and almost normal sensory hair cells. These results suggest that

1. Guinea pigs with immunologically induced endolymphatic hydrops can serve as a useful model of Meniere's disease.
2. Autoimmune response may play an important role in the etiopathogenesis of Meniere's disease.
3. Spiral ganglion cell degeneration, together with endolymphatic hydrops, may be responsible for abnormal changes in AP.

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THE INTERNATIONAL SYMPOSIUM ON CLINICAL AND BASIC OTOLOGY

The International Symposium on Clinical and Basic Otology will be held June 4-6, 1990, in Beijing, People's Republic of China. The abstract deadline is December 31, 1989. For further information, contact the Beijing Institute of Otorhinolaryngology, Chan Liu, MD, Professor and Director, 17 Hougou Lane, Beijing 1000005, People's Republic of China.

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The University of Iowa Department of Otolaryngology–Head and Neck Surgery and the International Cochlear Implant Association will hold the Second International Cochlear Implant Symposium in Iowa City on June 4-9, 1990. The symposium will focus on basic science aspects of electrical stimulation on the auditory system, new cochlear implant strategies, clinical application in both adults and children, and rehabilitation and educational issues. Please submit abstracts to Bruce J. Gantz, MD, Department of Otolaryngology–Head and Neck Surgery, University of Iowa Hospitals and Clinics, Iowa City, IA 52242.

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PROTEASES AND PROTEASE INHIBITORS IN IMMUNE COMPLEX OTITIS MEDIA IN CHINCHILLAS

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Using an immune complex-induced (IC) otitis media model in chinchillas sensitized with human serum albumin (HSA), we measured the levels of protease activity, protease inhibitors, and HSA in the middle ear fluid (MEF). The effect of a corticosteroid agent (triamcinolone) on the degree of IC otitis media also was studied. The levels of protease inhibitors α1-antitrypsin and α2-macroglobulin in the MEF were significantly higher than in nonsensitized animals. Fibrinolytic (plasminogen activator [PA]) activity was detected in the MEF; however, only a small level of activity of nonspecific proteases was detected because of the significant level of antitryptic activity in the MEF. The levels of protease inhibitors and PA activity were significantly reduced by the steroid treatment. It is concluded that protease inhibitors play an important role in the protection of the middle ear mucosa and that corticosteroid treatment can reduce the severity of IC otitis media.

KEY WORDS — human serum albumin, immune complex otitis media, middle ear fluid, protease inhibitor, steroid treatment.

INTRODUCTION

Otitis media with effusion (OME) is one of the most prevalent middle ear diseases. For better understanding of the pathogenesis of OME, experimental OME models in chinchillas have been used.[1,2] Recently, the significance of an immunologic reaction in the pathogenesis of OME has been proposed by many investigators,[3-5] and an immune complex-induced (IC) reaction seems to be responsible for the pathogenesis of chronic OME.[6] Models of OME induced by an antigen-antibody reaction have been studied by several investigators.[6-8] This reaction is characterized by remarkable leukocyte infiltration induced by complements and other chemotactic factors, and by tissue damage by lysosomal proteases released from leukocytes.[9] Proteolytic activity in the locus with IC inflammation is an important factor determining the degree of tissue damage, and it is controlled by protease inhibitors mainly derived from the blood.[10] Therefore, the balance between protease activity and inhibitor activity is significant in the pathogenesis of IC otitis media (IC-OM).

Using an IC-OM model in chinchillas sensitized with human serum albumin (HSA), we have measured nonspecific protease activity and the levels of protease inhibitors and HSA in middle ear fluid (MEF) recovered at different times after HSA instillation. The purpose of this study is to characterize the kinetics of the protease/inhibitor reaction during the course of IC-OM. The effect of a corticosteroid agent on the degree of IC-OM also was studied to obtain baseline information for possible local treatment with a steroid agent.

MATERIALS

Experimental procedures are summarized in Fig 1.

Nonsensitized Animal Group. Twenty-two chinchillas weighing 400 to 600 g were anesthetized with ketamine HCl (20 mg/kg, intramuscularly). One milliliter of HSA (5 g/dL; Sigma, St Louis) dissolved with sterile saline was instilled into the left middle ear bulla (MEB) of the normal chinchillas, and 0.5 mL of HSA (10 g/dL) containing the same volume of 25 mg/mL of triamcinolone diacetate (Aristocort, Lederle Parenterals, Carolina,

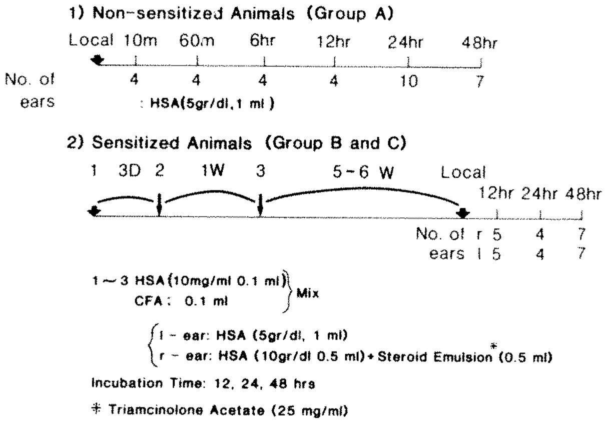
1) Non-sensitized Animals (Group A)
Local 10m 60m 6hr 12hr 24hr 48hr
No. of ears
4 4 4 4 10 7
: HSA(5gr/dl,1 ml)
2) Sensitized Animals (Group B and C)
1 3D 2 1W 3 5-6 W Local
12hr 24hr 48hr
No. of ears r 5 4 7
l 5 4 7
1 ~ 3 HSA(10mg/ml 0.1 ml) CFA: 0.1 ml Mix
l - ear: HSA (5gr/dl, 1 ml)
r - ear: HSA (10gr/dl 0.5 ml) + Steroid Emulsion* (0.5 ml)
Incubation Time: 12, 24, 48 hrs
* Triamcinolone Acetate (25 mg/ml)

Fig 1. Experimental procedures.

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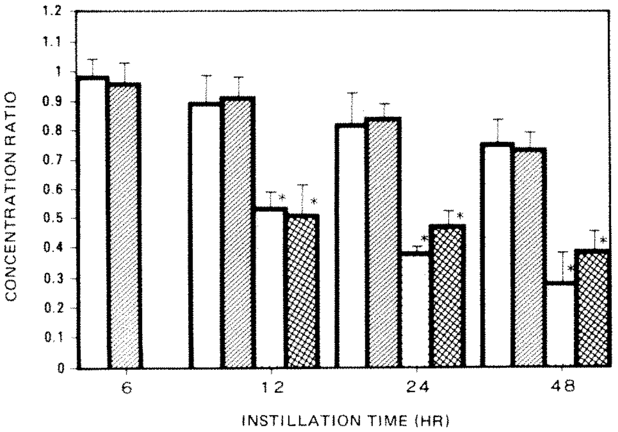

Fig 2. Human serum albumin (HSA) level (mean ± SD) in recovered middle ear fluid, expressed as ratio against 5 g/dL of HSA. ☐ — control group, ▨ — control + steroid group, ▒ — otitis media group, ▩ — otitis media + steroid group, V — significantly different from value at 6 hours after instillation (p< .01), * — significantly different from value of control group (p< .01).

Puerto Rico) was instilled into the right MEB. Middle ear fluids were recovered at 10 minutes and 1, 6, 12, 24, and 48 hours after instillation. The MEFs were centrifuged at 2,000 rpm for 10 minutes at 4°C. Blood samples were collected by cardiac puncture into a test tube containing 3.8% sodium citrate (1:10). After centrifugation, plasma samples were collected.

Sensitized Animal Group. Sixteen normal chinchillas were systemically immunized by 0.1 mL of HSA (10 mg/mL) containing the same volume of complete Freund's adjuvant (Sigma). The emulsion was injected subcutaneously under the back skin of the animals, which were boosted 3 days and 10 days after the first injection. When circulating antibody titers were elevated enough to induce local inflammation (2^4 to 2^6), 1 mL of HSA (5 g/dL) was instilled into the left MEB, and 1 mL of HSA containing 25 mg/mL of triamcinolone diacetate was instilled into the right MEB. The MEB was exposed surgically, and MEF retained in the MEB and a blood sample were collected at 12, 24, and 48 hours after instillation. Both MEF and plasma samples were centrifuged at 2,000 rpm for 15 minutes at 4°C and stored at −70°C until use.

METHODS

Quantitation of HSA in MEF. Concentrations of HSA were measured by electroimmunodiffusion (EID)[11] with a commercial goat anti-HSA antibody (Cooper Biomedical, Washington, DC). Its detection limit was 1 mg/dL. In order to avoid the cross reaction of chinchilla albumin against anti-HSA antibody, commercial antibody was preincubated with the same volume of normal chinchilla serum for 1 hour at 37°C. The MEF sample was diluted 100 times for this assay.

Quantitation of α1-Antitrypsin and α2-Macroglobulin Levels in Recovered MEF. Concentrations of α1-antitrypsin (α1-AT) in the recovered MEF were measured by single radial immunodiffusion,[12] and those of α2-macroglobulin (α2-M) were measured by EID with specific rabbit antibodies against purified chinchilla α1-AT and α2-M.[13]

Antitryptic Activity in MEF. Antitryptic activity (ATA) of free α1-AT was measured by Eriksson's method[14] and expressed as percent inhibition of hydrolysis of extrinsic trypsin.

Fibrinolytic and Nonspecific Protease Activity in Recovered MEF. Fibrinolytic (plasminogen activator [PA]) activity was measured by using 1% plasminogen-rich fibrinogen-agarose plate, and urokinase (Mochida, Tokyo) was used as a control.[15] The activity of PA was expressed in international units per milliliter of urokinase.

Nonspecific protease activity was measured by using 0.1% heat-denatured fibrinogen-agarose plate.[15] Its activity was expressed in the lytic area (mm^2). The detection limit was 5 μg/mL of trypsin (beef pancreas trypsin; Sigma).

Bacteriologic Study of Recovered MEF. After collection of the MEF, an aliquot of each sample was plated directly onto both sheep blood and chocolate agar plates for culture.

Measurement of Antibody Titer Against HSA in Plasma. Antibody titer against HSA was measured by the Ouchterlony method[16] and expressed in the maximum dilution ratio of plasma samples that made a precipitin line.

Statistical significance was determined by either Student's *t* test or a paired *t* test.

RESULTS

All the samples were divided into four groups: 1) control, 2) control + steroid, 3) IC-OM with instillation of HSA alone (OM), and 4) IC-OM with instillation of HSA containing triamcinolone diacetate (OM + steroid).

The bacterial culture test was negative for all the MEF samples.

HSA Levels in MEF. All the results are summarized in Fig 2. In both control and control + steroid groups, the HSA level decreased gradually, and the values at 24 and 48 hours after instillation were significantly lower than that at 6 hours after instillation (p< .01). In OM and OM + steroid groups, the HSA level decreased remarkably with the development of IC-OM, and the values at 12, 24, and 48 hours after instillation were significantly lower than those of the control group (p< .01). There were no significant differences between control and control + steroid groups or between OM and OM + steroid groups.

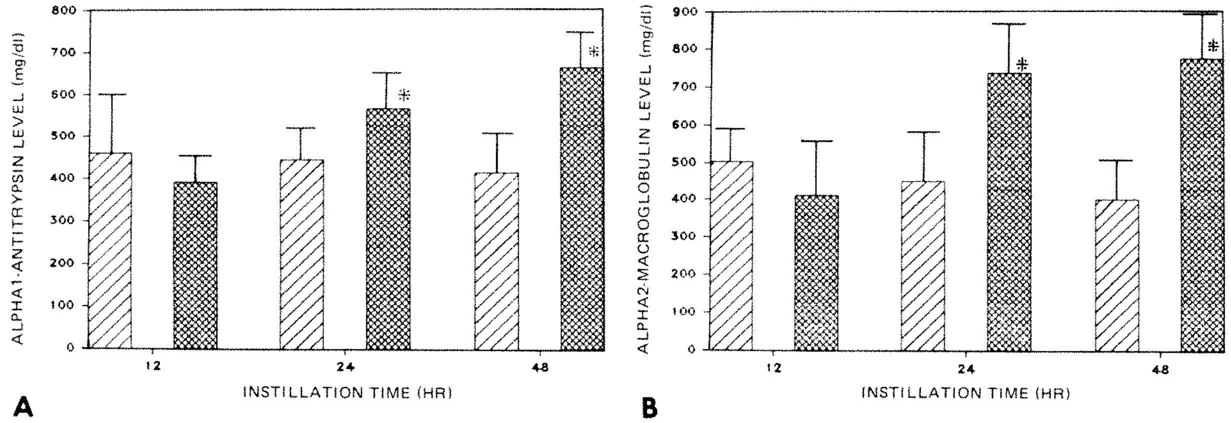

Fig 3. Levels (mean ± SD) of A) α1-antitrypsin (α1-AT) and B) α2-macroglobulin (α2-M) in plasma before (▨) and after (▩) instillation of human serum albumin (HSA). # — significant differences of both α1-AT and α2-M levels before and after instillation of HSA ($p<.01$).

Alpha1-Antitrypsin and α2-Macroglobulin Levels in Plasma and Recovered MEF. There was no significant difference in α1-AT or α2-M level in plasma before and 12 hours after instillation of HSA, whereas significant differences of both inhibitor levels were observed before and 24 and 48 hours after instillation of HSA ($p<.01$, paired *t* test; Fig 3).

The results of both α1-AT and α2-M levels in the MEF are shown in Fig 4. In both control and control + steroid groups, α1-AT in the MEF was detected 6 hours after instillation and reached its peak 24 hours after instillation. On the other hand, α2-M in the MEF was first detected 24 hours after instillation and then decreased. In the OM group, the α1-AT level increased remarkably after HSA instillation. The highest level of α1-AT was observed 48 hours after instillation (719.8 ± 146.1 mg/dL). In the OM + steroid group, the α1-AT level was significantly lower than that of the OM group (12 hours, $p<.025$; 24 hours, $p<.05$; and 48 hours, $p<.05$) and significantly higher than that of the control + steroid group (48 hours, $p<.01$). The level of α2-M in both OM and OM + steroid groups had a trend similar to that of the α1-AT level. The α2-M level in the OM + steroid group was significantly lower than that of the OM group (48 hours, $p<.01$) and significantly higher than that of the control + steroid group (12 and 48 hours, $p<.01$).

ATA in Recovered MEF. The results are summarized in Fig 5. The mean value of ATA increased gradually in both OM and OM + steroid groups after HSA instillation. There was a significant difference between the groups at 12 hours after instillation ($p<.05$). The level of ATA had no significant correlation with α1-AT level in the MEF samples of either group.

Proteolytic Activity of PA and Nonspecific Proteases in MEF. Fibrinolytic (PA) activity was not detected in any MEF sample of the control group from 10 minutes to 48 hours after instillation, but it appeared in the OM group at 48 hours (13.5 ± 8.9 IU/mL, which was significantly higher than that of

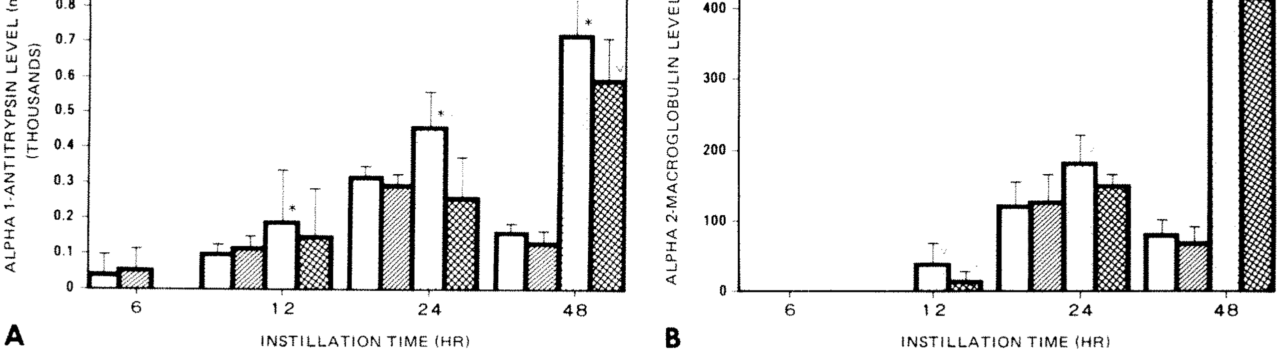

Fig 4. Levels (mean ± SD) of A) α1-antitrypsin (α1-AT) and B) α2-macroglobulin (α2-M) in recovered middle ear fluid. ☐ — control group, ▨ — control + steroid group, ▦ — otitis media group, ■ — otitis media + steroid group, * in A — significantly different from values of α1-AT in otitis media + steroid group ($p<.025$ at 12 hours, $p<.05$ at 24 hours, and $p<.05$ at 48 hours), * in B — significantly different from value of α2-M in otitis media + steroid group ($p<.01$ at 48 hours), > — significantly different from values of control group ($p<.01$).

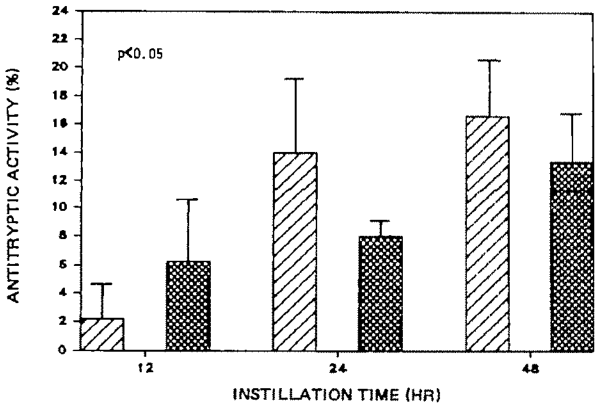

Fig 5. Antitryptic activity (mean ± SD) in recovered middle ear fluid. Significant difference could be observed between both otitis media (■) and otitis media + steroid (■) groups at 12 hours after instillation ($p < .05$).

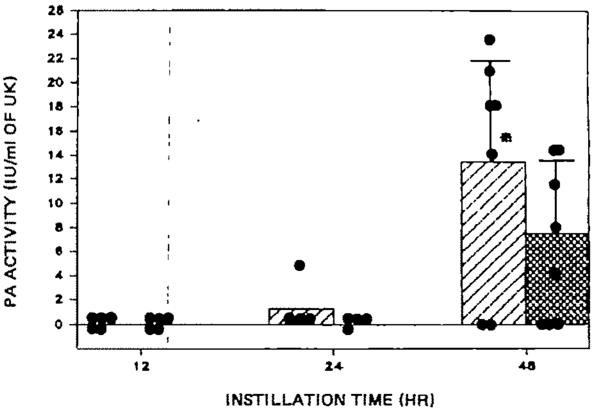

Fig 6. Fibrinolytic (plasminogen activator [PA]) activity (mean ± SD) in recovered middle ear fluid. UK — urokinase, ■ — otitis media group, ■ — otitis media + steroid group, * — significantly different from value of otitis media + steroid group at 48 hours after instillation ($p < .025$).

the OM+steroid group [$p < .025$]; Fig 6). The MEF samples in the four groups had no lytic area (activity) in the assay for nonspecific protease activity.

DISCUSSION

Human serum albumin, used as an antigen in this experiment, is a well-known plasma protein distributed widely in human tissues and body fluids. Fifty milligrams per milliliter of HSA instilled into the MEB is a concentration similar to serum albumin levels. A significant decrease of HSA level in the control group at both 24 and 48 hours after instillation may be attributed to the excretory function of the eustachian tube and the dilution effect by mild vascular leakage from the middle ear mucosa (MEM).[17] The HSA level in the MEF was significantly lower in both OM and OM+steroid groups than in the control group. In sensitized animals, instillation of a high concentration of HSA into the MEB induces a specific inflammation with an antigen excess condition against specific IgG in the middle ear, where soluble immune complex is formed. Immune complex is more rapidly phagocytized by leukocytes to be eliminated via both the lymphatic system and the eustachian tube, compared to free antigen,[18] contributing to the decrease of the HSA level in the OM group. A dilution effect by increased vascular leakage may also be related to this decrease.

Both α1-AT and α2-M are acute phase reactants in chinchillas,[19] which respond to systemic inflammatory changes. Therefore, significant increases of both α1-AT and α2-M levels in plasma at 24 and 48 hours after instillation of HSA suggest that IC-OM induces a systemic inflammatory reaction. Both α1-AT and α2-M are also major protease inhibitors derived from plasma and their levels are found to be very low in the normal middle ear.[13] Therefore, both inhibitors could be a useful index reflecting the vascular permeability (VP) of the MEM during inflammation. In the control group, the peak of both inhibitor levels was observed 24 hours after instillation, indicating that vascular leakage would take place and reach its maximum level about 24 hours after instillation. Alpha2-macroglobulin could not be detected within 12 hours of instillation because of its high molecular weight (840 kd). In both control and control+steroid groups, both the osmotic pressure effect and the nonspecific foreign body reaction of a high concentration (50 mg/mL) of HSA affect the middle ear mucosa,[17] leading to mild vascular leakage. In the OM group, both inhibitor levels increased after HSA instillation and were significantly higher than those of the control group. These results indicate that IC-OM developed after HSA instillation, enhancing the VP of the MEM. The VP in the OM group would be larger than that of the control group. Although we could not observe the peak of both inhibitor levels, previous study has shown that the peak of vascular leakage in an IC reaction is present a few days after antigen instillation.[20]

Both inhibitors (α1-AT and α2-M) complex with lysosomal proteases such as elastase, collagenase, and cathepsins to inhibit their activity, in an important protective function against tissue damage by lysosomal proteases. Alpha1-antitrypsin seems to occupy a larger portion of the inhibitory function in the MEF than α2-M, because the molar concentration of α1-AT is much higher. Antitryptic activity in the MEF was measured to estimate the level of inhibitory function of α1-AT. Antitryptic activity reflects the level of free α1-AT, while the α1-AT level measured by the present assay (single radial immunodiffusion method) reflects the levels of both free and complexed α1-AT. The level of ATA (free α1-AT) had no significant correlation with the α1-AT level in either the OM or the OM+steroid group. This suggests that the ratio of free to complexed α1-AT in the MEF would not be constant in both groups. Free α1-AT reacts with proteases to form protease–α1-AT complexes. The presence of free α1-AT and only a small activity of nonspecific proteases, namely elastase, cathepsin G, chymotrypsin, pronase, and plasmin,[15] in the MEF indicates the significance of inhibitory function of protease inhibitors against nonspecific proteases. Our previous study has shown that the presence of ATA in the middle ear effusion is an important factor in reducing mucosal damage in experimental bacterial otitis media.[19]

In the OM group, fibrinolytic (PA) activity was detected in the MEF at 48 hours after HSA instillation. Plasminogen activator is an enzyme converting proenzyme plasminogen into plasmin with fibrinolytic activity.[21] In general, fibrinolytic activity, mainly occupied by tissue type PA released from both vascular endothelium and infiltrating leukocytes,[21] becomes apparent in the locus about 24 hours after instillation of antigen.[20] In IC-OM, a similar trend was observed. A significant decrease of fibrinolytic activity in the OM+steroid group suggests that the release of tissue type PA from both vascular endothelium and infiltrating leukocytes may be reduced by steroid treatment.

To analyze the effect of a corticosteroid agent on IC-OM for possible clinical treatment, HSA containing a steroid emulsion (triamcinolone diacetate) was instilled into the MEB. Triamcinolone is a well-known corticosteroid used for the local treatment of chronic arthritis. The absence of a significant difference between control and control+steroid groups suggests that the effect of the steroid agent on the MEM is slight in the control group. However, the significant decreases of α1-AT, α2-M, and PA levels in the OM+steroid group indicate that triamcinolone affects the degree of IC-OM. Corticosteroid agents have various suppressing functions against allergic inflammations, such as inhibition of phospholipase A2 and the release of chemical mediators from leukocytes.[22] Inhibition of the production and release of vascular permeability-enhancing factors such as histamine, bradykinin, and arachidonic acid metabolites (prostaglandin E2, etc) may be related to the suppression of VP in the OM+steroid group. Although corticosteroid agents already have been applied to the treatment of chronic OME,[23] two major problems have to be resolved for possible clinical application in local therapy. The first one is the effect of steroid agents on OME associated with active bacterial infections, because the discrepancy between chronic OME associated with bacterial infections and IC-OM with sterile MEF is always present. The second one is the possibility of inner ear damage by steroid agents, because they can pass through the round window membrane into the inner ear.[24]

It is concluded that:

1. Human serum albumin (antigen) levels in the MEF decreased more quickly in the OM group than in the control group.
2. Immune complex-induced OM developed after instillation of antigen (HSA) into the MEB, and the levels of protease inhibitors α1-AT and α2-M were significantly higher in the OM group than in the control group.
3. Only slight activity of nonspecific proteases could be detected in the MEF because of the significant level of ATA of protease inhibitors, which is important for the protection of the MEM against proteolytic damage in IC-OM.
4. In the OM group, fibrinolytic (PA) activity appeared in the MEF about 48 hours after HSA instillation, suggesting the release of tissue type PA from the MEM and leukocytes.
5. Local treatment with a steroid agent (triamcinolone diacetate) significantly reduced the vascular permeability and fibrinolytic activity in IC-OM.

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IMAGING CASE STUDY OF THE MONTH

MAGNETIC RESONANCE IMAGING OF THORNWALDT'S CYST

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Magnetic resonance imaging has facilitated the ability of clinicians to diagnose Thornwaldt's cyst. Improved soft tissue contrast of MRI and additional imaging projections of MRI have increased the sensitivity and specificity in diagnosing this entity.

The soft tissues of the nasopharynx have a characteristic signal intensity on different spin echo T1-weighted images and T2-weighted images (Fig 1).[1,2] The brightest signal on T1-weighted images is fat, which fades on T2-weighted images. Both mucosa and lymphoid tissue (adenoids) have an intermediate signal. Muscles show the weakest signal intensity on both pulse sequences. Thornwaldt's cyst has a characteristic bright signal intensity on both T1-weighted and T2-weighted images (Fig 2) that is related to either its protein content or hemorrhage into the cyst.[2,3]

The availability of sagittal, coronal, and axial images with MRI helps to localize Thornwaldt's cyst to the mucosal space (Fig 2). The sagittal image demonstrates its location at the superior recess of the nasopharynx. Axial and coronal images localize

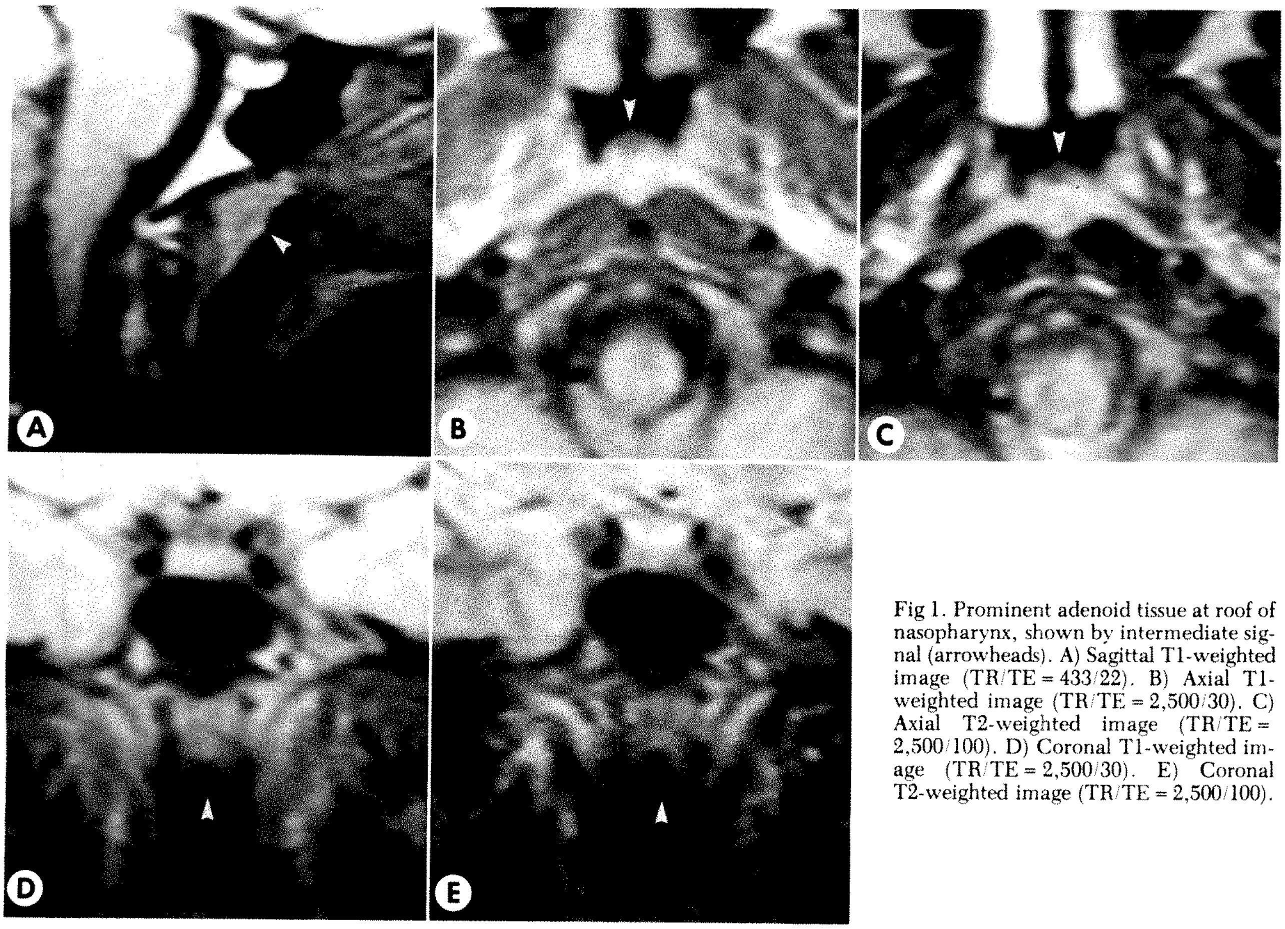

Fig 1. Prominent adenoid tissue at roof of nasopharynx, shown by intermediate signal (arrowheads). A) Sagittal T1-weighted image (TR/TE = 433/22). B) Axial T1-weighted image (TR/TE = 2,500/30). C) Axial T2-weighted image (TR/TE = 2,500/100). D) Coronal T1-weighted image (TR/TE = 2,500/30). E) Coronal T2-weighted image (TR/TE = 2,500/100).

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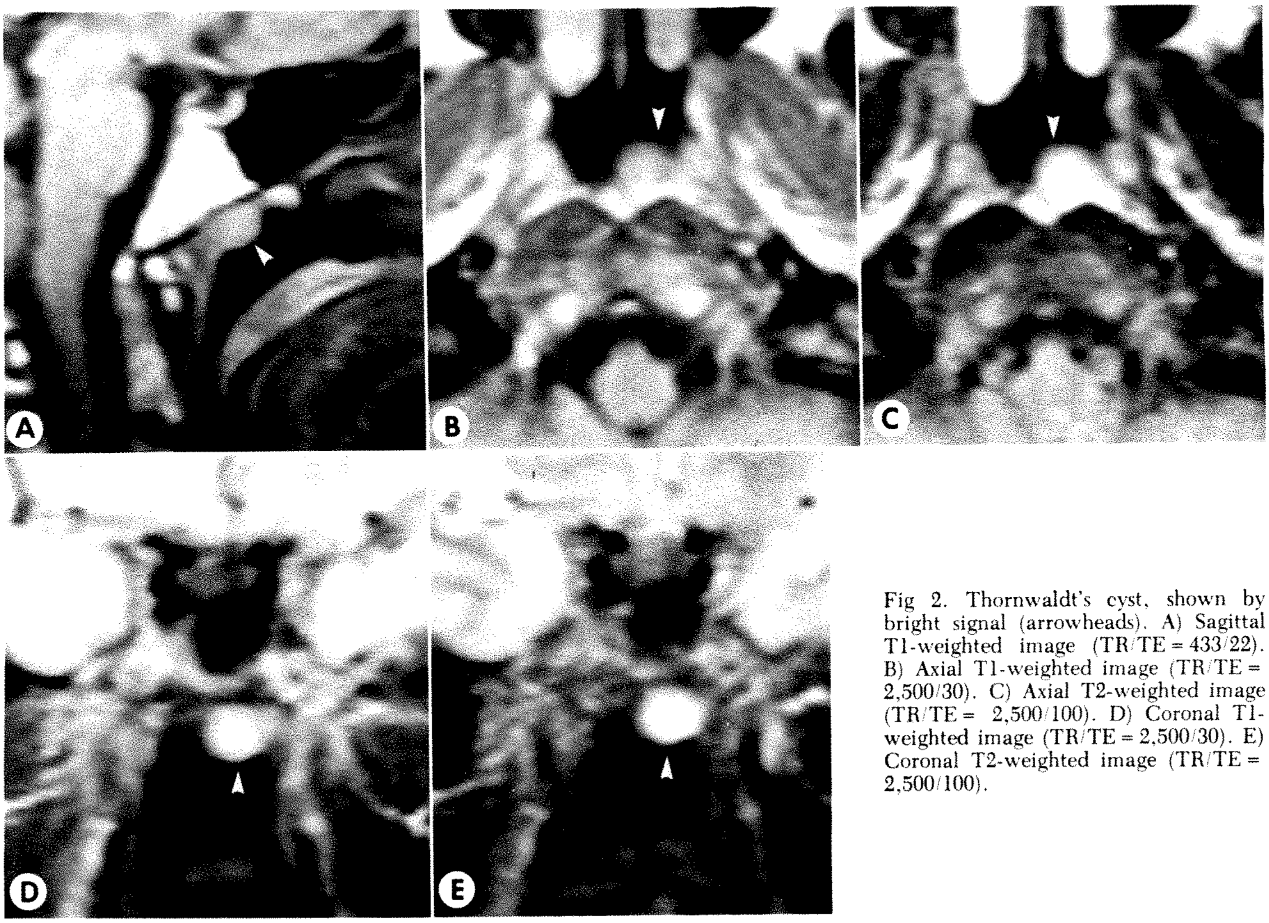

Fig 2. Thornwaldt's cyst, shown by bright signal (arrowheads). A) Sagittal T1-weighted image (TR/TE = 433/22). B) Axial T1-weighted image (TR/TE = 2,500/30). C) Axial T2-weighted image (TR/TE = 2,500/100). D) Coronal T1-weighted image (TR/TE = 2,500/30). E) Coronal T2-weighted image (TR/TE = 2,500/100).

its midline position. The multiple projections demonstrate that Thornwaldt's cyst has distinct margins, has the rounded shape of a cyst, and is limited to mucosa.

The characteristic location of Thornwaldt's cyst and the increased signal intensity identified on both T1-weighted and T2-weighted images helps to differentiate Thornwaldt's cyst from prominent adenoid tissue that is also located along the roof of the nasopharynx.

It is important to recognize the characteristic location of a Thornwaldt's cyst and the characteristic bright signal on T1-weighted and T2-weighted images. Patients with Thornwaldt's cyst can present with occipital headaches, pain and stiffness in the cervical muscles, ear pain, and nasopharyngeal drainage; therefore, a study of the brain, paranasal sinuses, nasopharynx, or neck may be requested.[4] The roof of the nasopharynx is included in these studies. Treatment will be delayed unless one realizes that the midline cystic mass at the roof of the nasopharynx is the cause of the patient's symptoms.

The differential diagnosis of a noninfected Thornwaldt's cyst is relatively limited because of its characteristic midline mucosal location and bright signal on T1-weighted and T2-weighted images. An infected Thornwaldt's cyst can present with nodes in the carotid and retropharyngeal spaces. This may raise a question of a nasopharyngeal carcinoma; however, the clinical presentation of pain and fever with a nasopharyngeal mass leads to a biopsy that will yield the correct diagnosis. Prominent adenoids do not have the characteristic bright signal on T1-weighted and T2-weighted images (Fig 1). Lymphoma, with involvement of the adenoids, presents with adenopathy and low signal intensity of the adenoids on T1-weighted images. Nasopharyngeal carcinoma does not have the bright signal intensity on T1-weighted images and may present with extension into the deep tissue spaces and adenopathy.

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PATHOLOGY CONSULTATION

CHONDROMYXOID FIBROMA

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Approximately 2% of chondromyxoid fibromas of bone present in the jaws, preponderantly in the mandible, where they must be distinguished from low-grade chondrosarcomas. A recurrence rate of 25% dictates careful complete removal of this histologically benign lesion.

Chondromyxoid fibroma comprises less than 1% of all bone tumors and is most often a lesion of the proximal metaphyseal ends of the long bones, particularly the tibia and femur.[1-4] Approximately 2% of all reported chondromyxoid fibromas have presented in the jaws, preponderantly the mandible, and skull bones.[1-3] At any osseous site, chondromyxoid fibromas most often occur in subjects who are in their second or third decades of life. In extragnathic sites, there is an equal sex distribution; females exceed males 2:1 for those in the jaws.[1]

Radiographs of the tumor suggest a benign lesion by a lobulated, circumscribed radiolucency with sclerotic and scalloped margins. The cortex of involved bone may be expanded or thinned and eroded, but the integrity of the periosteum usually is maintained.[1]

A chondroid character is conveyed by the tumor's gross appearance in that it is a firm yet compressible, white, somewhat translucent mass. Lobulation is a prominent feature, but the tumor is well circumscribed and has a generally quite distinct demarcation from bone. The tumor almost always is limited by an intact periosteum. Separate satellite nodules may be found rarely in adjacent bone.[3]

Histologically, the chondromyxoid fibroma has a considerable variability from tumor to tumor, as well as within areas of a single tumor. Chondroid and myxoid differentiation are the principal features, but a uniphasic preponderance may require a search for the other component. Lobules, usually fused and surrounded by a vascularized, nonneoplastic connective tissue, characterize the growth pattern (see Figure, A). The central parts of the lobules are usually myxoid, with stellate to spindle-shaped cells in a sparse distribution compared to the periphery, where there is typically a higher cell density. Chondroid zones tend to be less lobulated, and the intracellular matrix more homogeneous (see Figure, B). As the tumor matures, myxoid areas seem to undergo an increased collagenization. Besides its greater cellularity, the periphery also holds multinucleated giant cells and atypical-appearing nuclei in tumor cells (see Figure, C). Small amorphous calcifications and necrotic foci can be present in the tumor. Electron optic studies of chondromyxoid fibromas are few, but do indicate support for a cartilage origin for the tumors.[2]

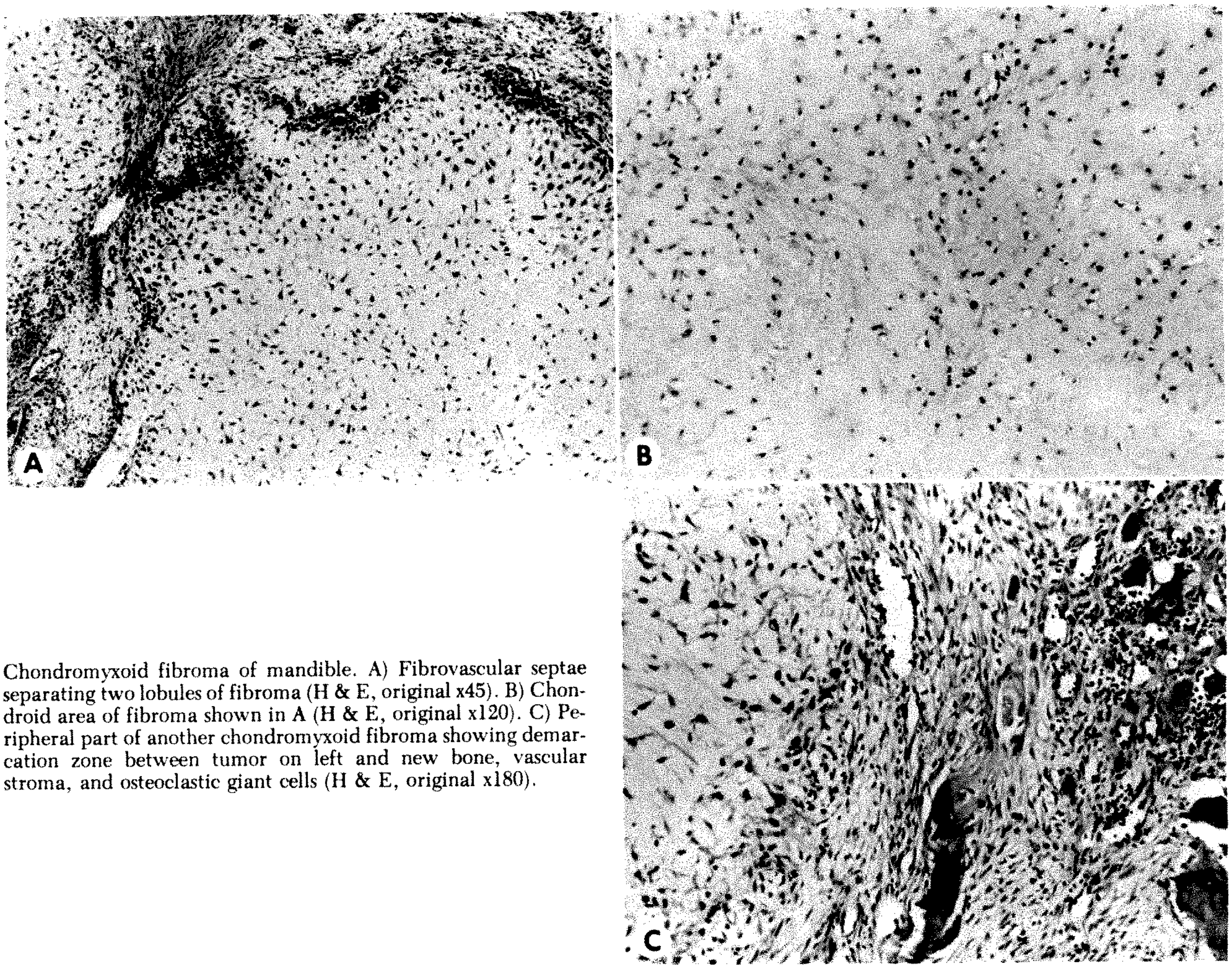
Chondrosarcoma is the most important, if not the most difficult, differential diagnosis for the surgical pathologist, who must correlate the findings with the radiographic presentation. Chondrosarcomas also may have prominent myxoid areas, scarce to absent mitotic figures, and a lobular pattern. They typically lack, however, the rather abrupt boundary and admixture of fibrous elements found in chondromyxoid fibromas.

Two different treatment modalities have been used, ie, en bloc resection or curettage, followed by immediate bone grafting.[1-3] Curettage has been followed by a recurrence rate of 25%, and an even higher rate in instances without bone grafts.[1,3] Failure of curettement has been attributed, in part, to incomplete removal of lobular extensions of the tumor, and liability of recurrence also appears favored in the more mucinous-myxoid tumors and in young patients.[3] Most recurrences appear within the first postoperative year. Radiotherapy is not effective, and in one patient was related to a posttherapy sarcoma.[4] In the gnathic bones, wide excision may be too radical a procedure, in view of potential cosmetic and functional deformities and since the posterior molar region and symphysis appear to be sites of predilection for this benign tumor. In such instances, thorough curettage with bone graft and follow-up is likely the wisest choice.[2]

Although malignant "transformation" has been mentioned by several authors, none of them adequately support the premise.[3,4]

From the University of Texas M. D. Anderson Cancer Center, Houston.

REPRINTS — John G. Batsakis, MD, Division of Pathology, University of Texas M. D. Anderson Cancer Center, 1515 Holcombe Blvd, Houston, TX 77030.



Chondromyxoid fibroma of mandible. A) Fibrovascular septae separating two lobules of fibroma (H & E, original x45). B) Chondroid area of fibroma shown in A (H & E, original x120). C) Peripheral part of another chondromyxoid fibroma showing demarcation zone between tumor on left and new bone, vascular stroma, and osteoclastic giant cells (H & E, original x180).

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SAN FRANCISCO OTOLOGY UPDATE: 1989

A course entitled San Francisco Otology Update: 1989, will be held November 2-4, 1989, at the Mark Hopkins Hotel in San Francisco. For further information, contact Extended Programs in Medical Education, University of California, Room U-569, San Francisco, CA 94143-0742; (415) 476-4151.

LETTERS TO THE EDITOR

To the Editor:

The association between blood transfusions and an increased rate of recurrence of head and neck cancer, described by Johnson et al[1] in 1987 and recently confirmed in this journal (Jackson RM, Rice DH; Blood Transfusions and Recurrence in Head and Neck Cancer. *Ann Otol Rhinol Laryngol* 1989;98:171-3), remains an unexplained phenomenon that deserves the urgent attention of surgical investigators.

In examining the data of Jackson and Rice we noted an inverse relationship of tumor stage to recurrence in patients receiving blood products. This unexpected, counter-intuitive finding raises a number of possible explanations. Unfortunately, several important factors that might have shed light on this matter were not analyzed.

Although blood transfusions appear to depress immunity,[2] it is not known nor is it clear whether the effect is due more to blood lost than to blood replaced or whether the effect is threshold related or dose related. It would be of interest to examine the relationship of recurrence rate to blood loss as well as to number of units replaced. Also, if immune depression is the mediator of the increased recurrence rate, one might expect different patterns of recurrence as well as time to recurrence in the transfused versus nontransfused groups.

Treatment factors strongly affect outcome and recurrence rate, yet radiotherapy and chemotherapy were not mentioned and surgical technique was not analyzed. Johnson et al[1] found an increased recurrence rate in transfused patients who had received postoperative radiotherapy, which suggests a possible interaction of the effects of transfusion and radiation upon the immune system. A comparison of the extent of surgical resection, the number and types of neck dissections performed, and the hemostatic techniques used by Jackson and Rice should have been discussed. One logically would expect that greater surgical skill would result in lower blood loss and better cancer eradication and conversely that more difficult dissections would result in more blood loss and greater opportunity for tumor dissemination.[3]

Finally, tumor-related factors, such as nodal reactivity, extranodal spread, degree of infiltration of tumor, preoperative weight loss, alcohol and tobacco use, which all affect or are affected by the immune system, were not evaluated. The absolute predominance of locoregional recurrence in this report, in contrast to other reports[4] in which distant metastasis is more common, creates additional problems in interpretation.

This is an important matter, one that demands an interinstitutional study to find answers to the many vital questions about the mechanism of this effect. If transfusion can be avoided by refinement in surgical technique, we, as head and neck surgeons, would have the opportunity for an immediate improvement in cancer control. We would be remiss if we did not pursue this promising avenue of clinical research.

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To the Editor:

The articles that Dr John Grow and I wrote 10 years ago concerning cricothyroidotomy[1,2] are well known to your readership. After our work was confirmed by other investigators, we thought that cricothyroidotomy had been discussed sufficiently and since then have focused our efforts on other issues.

Late in 1987, you published an article (Esses BA, Jafek BW; Cricothyroidotomy: A Decade of Experience in Denver. *Ann Otol Rhinol Laryngol* 1987;96:519-24) that concluded that cricothyroidotomy "has been severely restricted in Colorado, where it was popularized," a statement that is, at best, misleading. The authors' conclusions were based on a study of airway procedures carried out at the University of Colorado affiliated hospitals. Since our work was done primarily in the private community, and cricothyroidotomy has never been used to any extent at the University-affiliated hospitals, it's difficult to conclude that it's any less popular now than it was.

The use of illustrative cases is deceptive as well. Cases 1 and 2 sound similar to cases discussed in our 1982 article. In case 1, the bedside cricothyroidotomy was carried out because the patient had disseminated intravascular coagulation, she was in shock, and the nurses couldn't keep her endotracheal tube clear of blood. Cricothyroidotomy was a desperate procedure carried out on a patient who otherwise would have been considered to have contraindications to the procedure. The second patient had cricothyroidotomy carried out in the presence of acute laryngeal disease. His left recurrent laryngeal nerve had been injured by a stab wound and he had a hematoma of the oropharynx and nasopharynx. Thus, he had two contraindications for cricothyroidotomy. One also must question the adequacy of the diagnosis of his airway obstruction, as a 30% airway narrowing should not cause such severe symptoms. These patients are not typical candidates for cricothyroidotomy.

Our use of invasive airway procedures has changed substantially in the past decade primarily because of improvements in respiratory support technology. It is now uncommon in our practice for patients having elective cardiovascular surgery to require prolonged mechanical ventilation. Hence, the incidence of tracheotomy and cricothyroidotomy has decreased substantially. Our overall statistics remain the same, however. We have had no cases of chronic subglottic stenosis from cricothyroidotomy in the past 10 years, but we have had two cases of chronic subglottic stenosis resulting from standard tracheotomy. These data should not be construed as indicating that standard tracheotomy has a higher complication rate, as it has been our policy to carry out a standard tracheotomy in all patients who have acute laryngeal disease, and cricothyroidotomy in all others.

Drs Esses and Jafek are well aware that their study supports neither their claim for a higher incidence of complications nor restriction of use of the procedure in Colorado. Their careless scholarship tarnishes what otherwise would be a useful review article.

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To the Editor:

Thank you for the opportunity to respond to Dr Brantigan's letter. We will address individually each issue that he has raised.

In his first paragraph, Dr Brantigan stated that following "confirmation" of his and Dr Grow's work, cricothyroidotomy had been discussed sufficiently. No aspect of medicine should be closed to further discussion. In the case of cricothyroidotomy, we certainly are not alone in examining the procedure. All references in our literature review that questioned the procedure were published subsequent to Brantigan and Grow's original paper. The authors of these papers, and the many physicians in the United States and Europe who requested reprints of our article, do not feel cricothyroidotomy is a dead issue.

In his second paragraph, Dr Brantigan expresses concern over our extrapolation of the experience at the hospitals reviewed to the Denver experience. It is well agreed upon in epidemiology and biostatistics that in order to isolate a trend, one does not need to examine the population as a whole, but a representative sample. By using University Hospital, a tertiary care and private referral center; Denver General Hospital, one of the three hospitals in Denver designated as a trauma center; and the Denver VA Hospital, which has a five-state referral base, we felt that a representative sample had been studied. It should be noted also that during the study period, Dr Brantigan was on staff and had ample opportunity to introduce cricothyroidotomy to the University-affiliated hospitals. His observation is correct, however, that "cricothyroidotomy has never been used to any extent at the University-affiliated hospitals." We might add, for good reason: as cited in our paper, it carries an increased risk of subglottic stenosis and other complications.

Dr Brantigan then claims that the illustrative cases were deceptive as well. Dr Brantigan is basing this statement on his recollection, while our accounts were taken directly from the medical records. The medical record of case 1 did not describe the situation as desperate, nor did it mention disseminated intravascular coagulation. The patient was described as requiring long-term ventilatory management for pneumonia and hepatic coma. In case 2, the patient sustained stab wounds to the abdomen and chest and not to the neck. It was reported at one endoscopy following the cricothyroidotomy that the vocal cord was paralyzed. On subsequent endoscopies, the cord was found to be fibrotic and scarred, not paralyzed. The hematomas were in the nasopharynx and oropharynx by description; therefore, no acute laryngeal or subglottic trauma was present at the time of cricothyroidotomy. Granting the benefit of doubt that Dr Brantigan's recollections supplant the medical records, there is no mention of why cricothyroidotomy was carried out instead of tracheotomy, given the underlying contraindications. Even if the cases were as he describes them, our conclusion would remain the same: cricothyroidotomy should not be performed if laryngeal abnormality is present, and should be performed on an emergent basis only, with rapid revision to tracheotomy for prolonged ventilation.

Dr Brantigan further states, "Our overall statistics remain the same." We invite him to present these statistics with a comparable study group (1,000 patients).

Finally, we disagree with Dr Brantigan's indictment of "careless scholarship." Our report involved a precise scientific approach to reach a clear conclusion. Dr Brantigan accepts our report as a "useful review." Apparently, his major objection then is with the conclusions reached. While we respect his alternative interpretation of the data, we cannot accept it, and leave the final interpretation to the readers.

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To the Editor:

Cohen et al (Medical or Surgical Complications Related to the Nucleus Multichannel Cochlear Implant. *Ann Otol Rhinol Laryngol* 1988;97[suppl 135]:8-13) have pointed out that since the Nucleus multichannel cochlear implant has received premarketing approval from the Food and Drug Administration (FDA), "Reporting of complications to the manufacturer or the Food and Drug Administration is no longer mandatory (p 8)."

Although this is technically correct, the FDA strongly urges physicians to continue to report complications and adverse effects from this and other medical devices, even though the marketing of these products may have been approved by the agency. The clinical community is a vital source of information about the performance of devices under actual-use conditions; without such reporting, the FDA's ability to take timely action when needed (through recalls, for example, or labeling changes or user alerts) would be compromised seriously.

Physicians wishing to report complications or adverse effects from a medical device have two options. 1) Reports can be made to the manufacturer of the device. Federal law requires the manufacturer to report to the FDA when it becomes aware of information that its device may have caused or contributed to a death or serious injury. 2) Reports can be phoned directly to the FDA through its Product Problem Reporting Program, which is managed by the US Pharmacopoeia, by calling (800) 638-6725 or (301) 881-0256.

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BOOK REVIEWS

Investigating Hearing in Children

L. Fisch. Hard cover, illustrated, indexed, 273 pages, 1987. The Charles Press Publishers, Philadelphia, $29.95.

Doctor Fisch's stated purpose in writing this text is to provide "a broad, practical approach to audiological work with children" emphasizing the "practical aspects of hearing tests and general assessment of children." Drawing from more than 30 years of experience in pediatric audiology at various London facilities, he has accomplished his purpose.

This book is presented in a well-organized, easily readable fashion. The author is obviously a strong multidisciplinarian, even stating at one point that "audiology cannot be practiced properly by a single person...it must be the work of a multidisciplinary team." He then proceeds to list the members of such a team and describe the contributions of each professional specialist to pediatric audiology. While such a "team" approach is certainly optimal, in practice, facilities housing such a diverse group of professionals are very rare indeed. Although this text may be of interest to any one of the professionals on Dr Fisch's "team," in truth, some chapters would be of little practical value to anyone other than the audiologist or otologist because of the detailed technical discussion of such topics as the audiology testing center and the audiometer.

In stressing the importance of the audiologist's work after the testing itself has been completed, Dr Fisch points out the importance of striving to provide hearing-impaired children the "ability to communicate by speech" and "to enable them to cope with their disability as well as possible." He emphasizes that diagnostic work cannot be separated from training and rehabilitation, and that "a good hearing-aid service must be part and parcel of an effective audiology clinic." He goes on to say, "a scheme for early detection...is useless without a facility for diagnosis and assessment, and without proper facilities for treatment, parent guidance and education." However, he apparently feels that dealing with topics such as aural habilitation and amplification is beyond the scope of this text; therefore, this book deals primarily with the diagnostic aspects of pediatric audiology.

The importance of taking the case history of a hearing-impaired child is well discussed. There is also excellent material dealing with the child who is difficult to test, and on the importance of "calibrating the audiologist" in order to obtain a valid and useful audiogram. The author effectively describes various types of evaluations, including behavioral observation audiometry, conditioned response audiometry, play techniques, Bekesy audiometry (and its usefulness with children), and extensive coverage of pediatric speech audiometry.

A nice rationale is given for confirming any hearing loss detected by using more than one testing procedure. A few new test procedures ("toe test" and Oto Acoustic Emission Processor test) are introduced. The chapter entitled "Measurement of Middle-Ear Function" is a fine discussion of the importance of impedance testing of small children, on whom bone conduction audiometry cannot always be reliably performed. The information given about acoustic reflex testing and its diagnostic value in assessing facial nerve function is excellent as well.

Doctor Fisch gives a brief history of the development of "objective" tests, from psychogalvanic skin resistance to modern electrocochleography (ECoG). He devotes a good deal of time to transtympanic ECoG in comparison to relatively little about auditory brain stem evoked response (ABR) testing. In the United States, the ABR is probably much more widely used than the invasive ECoG, especially in children.

This text contains very clear, informative illustrations, is well indexed, and includes a very good glossary of terms. The appendices are devoted to the British standards for testing procedures and forms. One very nice addition is the inclusion of several case histories of hearing-impaired children representing a variety of situations and impairments.

Some readers might take issue with one or more of Dr Fisch's statements, however. For example, he states that "cochlear implants are still in an experimental stage." While this is true for children, in the United States the Food and Drug Administration has approved the implant for adults, and it is no longer considered "experimental." Further, he states that "it is unlikely that at present implanting electrodes into the inner ear (a major surgical procedure) is indicated in any hearing impaired child." There are some who feel that for a profoundly hearing-impaired child who cannot benefit from traditional amplification, the cochlear implant is a viable alternative.

Another point of possible contention is that in the case studies, the children are fitted with monaural hearing aids (if not exclusively, then at least initially), and parents are cautioned "never to force the use of the aid." A large number of professionals who deal with hearing-impaired children in this country believe that binaural hearing aids are essential for hearing-impaired children (except in the case of a unilateral loss, of course) for purposes of localization and improved performance in the presence of background noise (such as in a classroom). Also, with very young children who tend to throw their new hearing aids to the floor, it is necessary at times to enforce the use of the aid. Some children must be trained to use their aids just as they are trained to wear shoes or eyeglasses.

Overall, Dr Fisch's book offers a wealth of practical information and should prove to be a valuable, practical guide for anyone working in pediatric audiology. The very clinically oriented nature of the text makes it a good addition to the personal library of the professional engaged in this field.

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Otology Today

G. Babighian, editor. Volume 37 in *Advances in Oto-Rhino-Laryngology*. C. R. Pfaltz, editor-in-chief. Hard cover, illustrated, indexed, 199 pages, 1987. Karger, New York, $116.75.

This book offers 42 of the 60 papers presented at the Second International Workshop of Otology Today, held April 1-5, 1986, in Riva del Garda, Italy. It consists of four sections: basic science, diagnosis and clinical otology, otosurgery and otoneurosurgery, and hearing aids and cochlear implants.

The majority of the articles are found to be significantly lacking in scope and depth. Generally accepted policies and controversial issues are presented without adequate clarification. Many important issues are covered too superficially, lacking points of concern that are critical to the topic.

Two articles, on surgical treatment of cholesteatoma in children, and labyrinthine fistulas in cholesteatoma, present clear and concise reviews of the authors' experience while giving meaningful statistics and recommendations. Perhaps if the same criteria reflected in articles such as these had been applied to the rest of the book, the result would have been more worthwhile.

In general, this book does not present significant new information to the field of otology and, consequently, has limited application.

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Diseases of the Head and Neck: An Atlas of Histopathology

W. J. Arnold, J. A. Laissue, I. Friedmann, H. H. Naumann. Hard cover, illustrated, indexed, 330 pages, 1987. Thieme Medical Publishers, New York, $187.

The authors have compiled an extensive review of head and neck diseases and tumors in a single volume. The text is limited to defining each entity, providing a histopathologic description of each process, summarizing essential features, and listing differential diagnoses for most of the included diseases.

The atlas is well organized into 12 chapters, each focusing on an organ or anatomic region. Chapters are subdivided by disease processes into malformations and anomalies, infection and inflammatory changes, and benign and malignant neoplasms. In many instances, endoscopic photographs or radiographs are displayed alongside photomicrographs for clinical correlation. The outline style, combined with 39 tables, helps the reader collate and understand the complex pathology of the head and neck.

Excellent photomicrographs (most in color) and the high quality paper on which they are reproduced are the strength of this atlas. Essentially all of the diseases listed in the book are illustrated with well-selected photographs highlighting their main histopathologic features. Readers with less extensive training might have benefited from more thorough labeling of some of the pictures, but generally the legends and text are adequate. Curiously, melanoma is presented without discussion or illustration to give a concept of tumor thickness. These are minor omissions, and do not detract from the overall quality of the book.

Although this book provides no new information or insights, it does familiarize the specialist with the broad spectrum of disease found in this anatomic region. This concise and beautifully illustrated reference should serve well the busy practitioner needing a review, or the resident first encountering these diseases.

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Microscopy and Microsurgery of the Larynx and Laryngopharynx

Vanik S. Pogosov and Vasilii F. Antoniv (translated by Victor N. Bespalyi). Hard cover, illustrated, indexed, 180 pages. International Universities Press, Madison, Conn, $35.

This treatise on otolaryngology by two eminent Soviet physicians has recently been made available in English translation. Their emphasis is on surgical techniques based upon their combined clinical experience and observations on over 2,600 patients at a major Soviet hospital. The date of the original publication is not noted on the book.

The introduction includes a brief historical review of laryngoscopy and microlaryngeal surgery. In addition, there are chapters on instrumentation and the differential diagnosis for benign and malignant laryngeal disease. The largely descriptive text draws on the authors' personal experience in laryngeal pathology and microsurgical techniques during their career. Relatively simple microsurgical techniques for excision of benign lesions (eg, vocal cord stripping) are demonstrated and applied to early "non-invasive malignant disease." Their approach is straightforward and direct.

Interspaced within the book are specific case histories, eg, papillomatosis and squamous cell carcinoma of the larynx. Surgical procedures are illustrated by simple, unsophisticated drawings. The book lacks depth in its discussions and is dated, since references or techniques arising after 1979 are not included. Specifically, there is virtually no discussion of the use of carbon dioxide laser for laryngeal tracheal surgery.

Although handsomely printed, the illustrations are simplistic and the photographs are of mediocre quality. A few tables are included as a compilation of the authors' clinical experience.

The level of discussion is directed toward residents at an early level of training or medical students. I think this book would appeal only to those people interested in Soviet medicine. I do not recommend this book for otolaryngologists who want to maintain and devote their time to state-of-the-art procedures.

JAMES S. REILLY, MD
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The Larynx

Harvey M. Tucker. Hard cover, illustrated, indexed, 311 pages, 1987. Thieme Medical Publishers, New York, $94.

This is a well-written, nicely organized, carefully edited book by a physician and surgeon who has achieved national and international distinction in the study of the larynx and its disease. It is fitting that this author should have devoted his attention to the creation of a text dedicated to the subject of laryngology. It is the intention of the author in his preface to posture himself as an educator and to direct the emphasis of the text to students and residents.

Throughout the writing of this text, which was with a small number of well-known contributors, the format used was one in which margin notes were used to quote a well-known authority, or to point out the possible differences of opinion and highlight this to the reader, without detracting from the flow of thought in the actual text itself. By these means, the author has achieved a very readable and concise text.

The text is divided into 13 chapters. Chapter 1 details the anatomy and embryology of the larynx, with excellent black-and-white and color illustrations. Chapter 2 directs the reader's attention to the physiology of the larynx. This particular subject in many textbooks tends to be verbose and complicated. Dr Tucker has mastered the important details of laryngeal physiology and has presented them in a very understandable format. The illustrations are artistically excellent and embellish the text.

Chapter 3, authored by Hyams and Heffner, is devoted to laryngeal disease. This is an excellent, concise, and well-illustrated description, covering all aspects from benign to malignant neoplasia.

Doctors Noyek and Shulman author Chapter 4, which is devoted to diagnostic imaging of the larynx. This chapter is particularly well written and documented with an excellent bibliography for the student or resident interested in pursuing this area of expertise in greater detail.

Chapter 5 is devoted to the production of voice and the identification and evaluation of vocal pathologic states. This chapter, which is authored by Melinda Harrison and co-authored by Dr Tucker, is clear, concise, and well documented.

Chapters 6 through 13 have been written basically by Dr Tucker himself. Beginning with congenital disorders of the larynx and concluding with malignant tumors of the larynx, these chapters discuss important areas of laryngology. Included is an excellent section on the use of diagnostic surgical techniques in laryngology, such as laryngoscopy and endoscopic sinus surgery. Each chapter has an excellent bibliography. Illustrations are artistically clear and contribute to the discussion in the adjacent text.

In summary, the masterful use of margin notes; clear, concise textual writing; and marvellous illustrations have resulted in what I consider to be an overall outstanding textbook of laryngology. The extensive bibliography accompanying each chapter is in itself worth the price of the text. This book will undoubtedly be useful as a teaching and reference guide for a predominantly subspecialist audience in otorhinolaryngology, and is clearly written with the student and resident in mind. I would also recommend this text to other subspecialties that interface with otolaryngology, such as pulmonary medicine, general plastic surgery, and head and neck surgical oncology. It is also a good choice for speech pathologists and nurse practitioners interested in achieving an educational background in laryngology.

RICHARD L. FABIAN, MD
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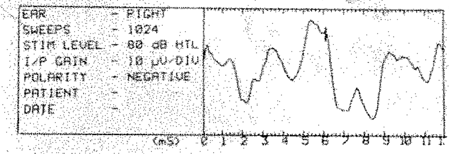

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